ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 795, 798 and 799

[OPTS-42115, FRL 3795-7]

RIN NO. 2070-AB07

Brominated Flame Retardants (Group I); Proposed Test Rule

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: EPA is issuing a proposed test rule under section 4(a) of the Toxic Substances Control Act (TSCA) in response to the Interagency Testing Committee (ITC) designation of the following five brominated flame retardants (BFRs) for health and environmental effects and chemical fate testing: (1) pentabromodiphenyl ether (PBDPE; CAS. No. 32534-81-9), (2) octabromodiphenyl ether (OBDPE; CAS. No. 32536-52-0), (3) decabromodiphenyl ether (DBDPE; CAS No. 1163-19-5), (4) 1,2,bis(2,4,6-tribromophenoxy)ethane (BTBPE; CAS. No. 37853-59-1), and (5) hexabromocyclododecane (HBCD; CAS. No. 3194-55-6). EPA has concluded that: activities involving these BFRs may pose an unreasonable risk of injury to human health or the environment as suggested by certain preliminary data; existing data are inadequate to assess the risks to human health and the environment posed by exposure to these substances, and testing of each of the five BFRs is necessary to develop such data.

DATES: Submit written comments on or before August 26, 1991. If persons request an opportunity to submit oral comment by August 9, 1991, EPA will hold a public meeting on this proposed rule in Washington, DC. For further information on arranging to speak at the meeting see Unit VIII of this preamble.

ADDRESSES: Submit written comments, identified by the document control number (OPTS-42115), in triplicate to: TSCA Public Reading Room (TS-793), Office of Pesticides and Toxic Substances, Environmental Protection Agency, rm. NE-G004, 401 M St., SW., Washington, DC, 20460.

A public version of the administrative record supporting this action, without confidential business information is available for inspection at the above address from 8 a.m. to 12 noon, and 1 p.m. to 4 p.m., Monday through Friday, except legal holidays.

FOR FURTHER INFORMATION CONTACT: David Kling, Director, Environmental Assistance Division (TS-799), Office of Toxic Substances, rm. E-543B, 401 M St., SW., Washington, DC 20460, (202) 554-1404, TDD (202) 554-0551.

SUPPLEMENTARY INFORMATION: This document proposes a test rule to require certain health, environmental, and chemical fate tests for the following five brominated flame retardants:

chemical substance	CAS No.	Docket No.
pentabromodiphenyl ether (PBDPE)	32534-81-9	42115/42145
octabromodiphenyl ether (OBDPE)	32536-52-0	42115/42146
decabromodiphenyl ether (DBDPE)	1163-19-5	42115/42147
1,2,bis(2,4,6-tribromophenoxy)ethane (BTBPE)	37853-59-1	42115/42148
hexabromocyclododecane (HBCD)	3194-55-6	42115/42149

The proposed health effects testing consists of tiered mutagenicity testing (all), subchronic toxicity testing (HBCD), neurotoxicity testing (all), reproductive effects testing (all), developmental toxicity testing (all), chronic toxicity testing (PBDPE, OBDPE and BTBPE), and oncogenicity testing (PBDPE, OBDPE, BTBPE, and HBCD).

The proposed environmental effects testing consists of the algal assay (all), fish early life stage toxicity testing (all), aquatic invertebrate chronic toxicity testing (all), benthic organism toxicity testing (all), mallard reproduction testing (all), laboratory earthworm testing (all), terrestrial plant testing (all), immunotoxicity testing (all), and bioconcentration testing (all).

The proposed chemical fate testing consists of testing to determine vapor pressure (all), water solubility (all), log octanol/water partition coefficient (PBDPE, OBDPE, and DBDPE), direct and indirect photolysis (all), biodegradation testing in water/sediment (all), sediment and soil adsorption (all), and anaerobic biodegradation (all). Testing is conditional for all environmental testing and the biodegradation testing in water/sediment for two BFRs (OBDPE and DBDPE) based on results from PBDPE.

I. Introduction

A. ITC Recommendation

The ITC designated the chemical category "brominated flame retardants" for chemical fate, health and environmental effects testing. The reasons for this designation are discussed in the Federal Register of December 12, 1989 (54 FR 51114).

B. Test Rule Development Under TSCA

EPA has evaluated the ITC's testing recommendations for the BFRs, relying heavily on the Information Review (Ref. 1) developed by the ITC, as well as the supplemental information developed by EPA. On the basis of this evaluation, EPA is proposing chemical fate, health effects and environmental effects testing for the BFRs under TSCA section 4(a)(1)(A). A discussion of the TSCA section 4 findings was provided in the Federal Register of July 18, 1980 (45 FR 48524). EPA is not now making findings under section 4(a)(1)(B) because EPA is developing its response to the court that remanded a test rule promulgated under TSCA section 4(a)(1)(B) for cumene (In *Chemical Manufacturers Association et al. v. Environmental Protection Agency* (899 F.2d 344 (5th Cir. 1990)). EPA

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reserves the right to make findings for BFRs under TSCA section 4(a)(1)(B) in the future.

This action constitutes EPA's response to the ITC as required by TSCA section 4.

II. Review of Available Data

A. Profile

The ITC (Ref. 1) designated five BFRs for priority testing. Three, PBDPE, OBDPE, and DBDPE, are structurally similar and are placed in a single category for some testing purposes. BTBPE and HBCD, while sharing similar uses with the three diphenyl ethers, are structurally dissimilar to them and to each other, and therefore are considered individually with respect to testing. All of these BFRs are solids at room temperature and have relatively low water solubility (Ref. 1).

B. Production and Use

Specific production volumes of each BFR have been claimed as confidential business information (CBI).

The BFRs are used mainly as additives to various plastic resins to impart resistance to burning. BFRs are primarily used in polystyrene, ABS resins, and epoxies. HBCD is used in polystyrene foam, and BTBPE in ABS resins and unsaturated polyester (Ref. 1).

C. Exposure and Release

Environmental releases and exposures of humans are anticipated from manufacturing and processing and from packaging and cleaning operations associated with the production and use of these BFRs (Ref. 1). EPA estimates that 160 to 2,200 workers may be exposed to the 3 diphenyl ethers through the inhalation and dermal routes (Ref. 10). No estimates were available for BTBPE or HBCD. PBDPE, DBDPE, and BTBPE were detected in air and soil near two U.S. production facilities and PBDPE has also been detected in fish, marine mammals, and birds in Sweden and in mussels and river sediment in Japan (Ref. 1). These detections are relevant to general population and environmental exposures. DBDPE was also found in shellfish and sediments in Japan (Ref. 1). BFRs, including PBDPE, have recently been detected in Atlantic bottle-nosed dolphins on the U.S. East Coast (Refs. 1 and 3). Although EPA is not aware of any reports that OBDPE and HBCD have been detected in the environment, they have uses similar to the other three BFRs and can reasonably be anticipated to be similarly released to the environment.

In an analysis of human adipose tissue from the fiscal year (FY) 1987 National Human Adipose Tissue Survey specimen repository, nearly all of the adipose tissue extracts analyzed contained hexa- through octabrominated diphenyl ethers (Ref. 4). The analytic methodology did not permit brominated diphenyl ethers with fewer than six bromines to be detected. Exact tissue levels were also difficult to measure, but approximate levels, from 5 to 8,000 picograms/gram (pg/g), were measurable from the composite samples. The National Human Adipose Tissue Survey Specimen repository represents a more or less random sampling of the general population. These data indicate exposure to the BFRs is widespread, and may also indicate that these substances have potential to bioaccumulate (i.e., the uptake and subsequent accumulation of a substance in an organism's tissues either through direct (e.g., respiration) or indirect (e.g., food consumption) means) in the human population.

D. Health Effects

1. *Metabolism and pharmacokinetics.* In a metabolism study in male rats with radiolabelled DBDPE, administered at 250 to 50,000 ppm in the diet, the majority of the compound was, after 9 to 11 days, excreted in the feces (82 to 100 percent) with a small amount (< 0.012 percent) excreted in the urine (Ref. 1). Most of the compound was excreted unchanged, with small amounts of three unidentified metabolites also detected. When administered as a single dose by gavage, similar results were obtained.

BTBPE also appears to be poorly absorbed through the gut. When radioactive BTBPE was administered to rats, 80 percent was recovered in the feces, and 5 percent was recovered in the urine within 4 days of dosing (Ref. 1).

2. *Acute and subchronic effects.* All of these BFRs are of low acute toxicity. The oral LD50 in rats for PBDPE was 7,400 mg/kg males and 5,800 mg/kg for females. LD50 values were not determined for OBDPE or DBDPE, but would exceed the 5,000 mg/kg administered (Ref. 1). Similarly, 10,000 mg/kg administered as an acute oral dose was insufficient to provide an LD50 value for BTBPE and HBCD (Ref. 1).

Subchronic studies have yielded a lowest observed adverse effect level (LOAEL) of 10 mg/kg/day for OBDPE (90-day dietary study), and liver effects were also seen at all doses tested (100, 1,000, and 10,000 ppm) in a 90-day oral gavage study for OBDPE. Similarly, in a 14-day study (inhalation of OBDPE as a dust) hepatocellular enlargements and necrosis were observed at all doses tested, 12, 120, and 1,200 mg/m^3 (Refs. 1 and 5). A no observable adverse effect level (NOAEL) of 8 mg/kg/day was obtained for DBDPE in a 30-day dietary study (Ref. 1). PBDPE, tested in a 90-day dietary study in rats with a subsequent 24-week follow-up period, caused irreversible liver hyperplasia at 2 and 100 mg/kg/day; a NOAEL was not established. Reversible thyroid hyperplasia was also observed (Refs. 1 and 5). In another 90-day study in rats a LOAEL of 10 percent of the diet (about 5,000 mg/kg/day) and a NOAEL of 1 percent in the diet were established for BTBPE (Refs. 1 and 5).

Liver effects, including enlarged liver cells and/or hepatocellular lesions were common to all of these chemicals (Refs. 1 and 5). Furthermore, the diphenyl ether compounds all showed thyroid hyperplasia (Refs. 1 and 5). In the acute studies with PBDPE, tremors and reduced activity immediately after exposure were also observed (Ref. 1).

3. *Chronic effects.* Chronic data were developed for DBDPE in two separate studies. In a 2-year feeding study done by the National Toxicology Program (NTP), the NOAEL was >2,240 mg/kg/day in rats, the highest dose tested. In mice, there was a doserelated thyroid hyperplasia observed at the 3,200 and 6,400 mg/kg/day dose (Refs. 1 and 6). A 2-year feeding study in rats conducted by Kociba et al. (1975) at much lower doses saw no effect at 1 mg/kg/day, the highest dose tested (Refs. 1 and 7).

4. *Oncogenicity.* Only DBDPE has been examined for oncogenic potential. DBDPE administered to rats at doses of 0.01, 0.1, or 1.0 mg/kg/day for 2 years showed no evidence of oncogenicity (Ref. 1). However, another bioassay performed by NTP, which was specifically designed to determine oncogenic potential, found oncogenicity expressed in both male and female rats. There was also some evidence of oncogenicity in male mice, but no evidence in female mice (Ref. 7). Dose levels in the NTP study were targeted at 25,000 and 50,000 ppm in the diet (approximately 1,250 and 2,500 mg/kg/day). Specific lesions in the form of neoplastic nodules were noted in the liver of the male and female rats and hepatocellular carcinomas or adenomas in male mice. DBDPE has, as a result of this study, been classified as a possible human carcinogen, class C (Ref. 5).

5. *Mutagenicity.* As reported by the ITC, mutagenicity testing performed to date has been negative for all five of these substances. Ames *Salmonella* testing was completed, with and without activation, for all five substances. A *Saccharomyces* assay for OBDPE was

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also done (Ref 1). Beyond this, additional testing has been done only for DBDPE, consisting of an *in vitro* ytogenetic assay in Chinese hamster ovary (CHO) cells, an *in vitro* sister chromatid exchange assay, a mouse lymphoma assay, and an *in vivo* study in rats, examining rat bone marrow cells. DBDPE gave no evidence of mutagenicity in these tests (Ref. 1).

6. *Developmental toxicity.* OBDPE was administered by gavage to 10 rats per dose group at doses of 2.5, 10, 15, 25, or 50 mg/kg on days 6 through 15 of gestation. The results were reduced ossification, a decrease in mean fetal weight, and an increase in post-implantation losses in the high-dose group. The NOAEL was 2.5 mg/kg/day, while the LOAEL was 10 mg/kg/day, based on decreased fetal weight (Refs. 1 and 5). The observed toxic effect on the offspring was attributed by the study investigators to maternal toxicity, which was observed at the high dose level.

DBDPE when administered to rats at doses of 10, 100, or 1,000 mg/kg showed no statistically significant developmental toxicity. However, there was an increase in subcutaneous edema and delayed ossification in the fetuses, with effects seen even at the lowest dose (10 mg/kg) tested (Refs. 1 and 5).The ITC also reported that BTBPE, tested at doses from 30 mg/kg to 10,000 mg/kg in rats, and HBCD administered to rats at 0.01, 0.1, or 1 percent of the diet (high dose approximately 500 mg/kg) during days 0 to 20 of gestation, showed no developmental effects (Ref. 1).

7. *Reproductive effects.* Only DBDPE, of these five substances, has been tested for reproductive effects. In a single-generation study, DBDPE was administered to rats at doses of 3, 30, or 100 mg/kg for 90 days prior to mating and through lactation. DBDPE had no effects on the offspring of these rats (Ref. 1).

8. *Neurotoxicity.* No neurotoxicity testing has been performed for any of these substances. However, acute studies on PBDPE saw diminished motor activity in rats during 1-hour exposures by inhalation to concentrations up to 200 mg/L (about 4.8 mg/kg). In another PBDPE study in rats, forelimb tremors and reduced motor activity were observed at an oral dose ≧ 4,000 mg/kg, but not at the lower doses (Ref. 1).

E. Environmental Effects

1. *Acute and short-term effects.* Acute toxicity is usually determined by exposing test organisms for a relatively short period (e.g., 48 or 96 hours). To see the measured effect (usually lethality) the doses used must normally be much higher than those required to exert an often more subtle effect (e.g., decreased reproduction or growth) looked for in a longer-term, chronic test. For these BFRs, determining their acute toxicity is problematic. Limited aquatic toxicity data indicate that their acute toxicity values exceed their (very low) water solubility, obfuscating interpretation of the results. The EC50 of DBDPE to algae was >1 mg/L (Ref. 1). This value greatly exceeded DBDPE's water solubility, determined by Norris (1974) to be between 0.02 and 0.03 mg/L (Refs. 1 and 8). As reported by the ITC, BTBPE LC50 values for bluegill, rainbow trout, and killifish were 1,531, 1,410 and 230 mg/L, respectively (Ref. 1). An algal study with HBCD by Walsh et al. (1987) gave an EC50 between 0.01 and 0.14 mg/L, indicating high toxicity to algae but still exceeding HBCD's reported water solubility of 0.008 mg/L (Refs. 1 and 9). The highest treatment concentration of a toxicity study should not exceed the aqueous solubility limit of the chemical. Ambient concentrations of chemicals rarely, if ever, exceed the aqueous solubility limits.

2. *Chronic toxicity and bioconcentration studies.* No chronic studies were found for any of these BFRs. Bioconcentration factors (BCFs) were determined for PBDPE and OBDPE by exposing carp to each of these chemicals for 8 weeks. The BCFs were 5,380 for carp exposed to PBDPE at 105 μg/L, and 11,700 when the water concentration was 9.7 μg/L. Using the same methodology, the BCF for OBDPE was ≦ 3.8 (Ref. 1). In a nonstandard bioconcentration test, Norris et al. (1974) found that when rainbow trout were exposed to 20 μg/L DBDPE for 48 hours, the fish contained only 6 μg/L at the end of the test period, which may indicate slow uptake (Refs. 1 and 8).

Carp were also exposed to 0.27 or 0.026 mg/L BTBPE for 8 weeks. For these two exposure concentrations, the respective bioconcentration factors in carp were 27 and 43 (Ref.1).

No elimination half-lives were reported for any of these bioconcentration studies.

F. Chemical Fate

Limited chemical fate information was available for the BFRs. Water solubility values estimated or determined for these BFRs are 0.6 ppb (PBDPE), 20 to 30 ppb (OBDPE and DBDPE), 200 ppb (BTBPE), and 8 ppb (HBCD) (Ref. 1). Octanol/water partition (Log K_{ow}) coefficients, which are negatively correlated with water solubility, are given as 7.8 (PBDPE), 5.5 (OBDPE), 5.24 (DBDPE), 3.14 (BTBPE), and 5.81 (HBCD) (Ref. 1). The ITC also reported that for the three biphenyl ether compounds vapor pressures are estimated to be less than 10^{-6} mm Hg (Ref. 1). BTBPE and HBCD should have similarly low vapor pressures. From these factors, low water solubility, high Log K_{ow} values and low vapor pressure, the ITC anticipated that the BFRs are likely to partition to soil, sediments, and biota (Ref. 1). However, even though these compounds may have low vapor pressures, their very low water solubility means that they may volatilize from water and soil/sediments and hence also partition to the atmosphere.

There is little other fate information. Shake-flask biodegradation of BTBPE showed that BTBPE is degraded, although indicating slow rates; and an aerobic study showed that HBCD might be degraded under certain conditions (Ref. 1). Norris et al. (1974) found that DBDPE could be degraded by photolysis, although no rates of photolysis were reported. Similarly, BTBPE was degraded when exposed to ultraviolet (UV) light (Refs. 1 and 8).

III. Findings

EPA is basing its proposed testing of PBDPE, OBDPE, DBDPE, BTBPE, and HBCD on the authority of section 4(a)(1)(A) of TSCA. EPA considers these findings to be sufficient for the testing proposed in this rule. However, as noted in Unit I.B. of this preamble, EPA reserves its right to also make findings under TSCA section 4(a)(1)(B) in the future for these BFRs.

Under TSCA 4(a)(1)(A), EPA finds that the manufacturing, processing, distribution in commerce, use, or disposal of BFRs may present an unreasonable risk of injury to health and to the environment.

Although there were mixed results, available data indicate that these BFRs may have the potential to exert developmental toxicity effects as described in Unit II.D.6. of this preamble. EPA also believes that these BFRs may have the potential to bioaccumulate in animal tissues, in which case the full expression of their general toxicity may be missed in a test less than a full chronic assay.

Available data further indicate that DBDPE is a potential human carcinogen, as shown by positive oncogenicity results in rats and mice in a 2-year bioassay performed by the NTP (Ref.1). EPA also notes that PBDPE and OBDPE are structurally similar to DBDPE and may, therefore, also be potential human oncogens. Furthermore, the three diphenyl ethers and BTBPE are similar in structure to certain polychlorinated dibenzo-*p*-dioxins (PCDDs),

polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs) that have been found to be carcinogenic in animal testing. One chemical which EPA finds structurally similar to the BFRs, tetrachlorobenzo-*p*-dioxin (TCDD), is also a potent immunosuppressor in several species of mammals (Ref. 11). Immunosuppression may be a mechanism enhancing tumor development (Ref. 11).

Immunosuppression is also an important toxicological endpoint in itself, leading to decreased disease resistance. In recent years major dolphin kills have occurred in the United States and Europe. Many of the dolphins found dead or dying were marked by the presence of BFRs, including PBDPE, in their tissues. Suppressed immune function was also seen. Although the putative cause of these deaths is toxic algal blooms ("red tide"), which may also cause immune suppression (Ref. 13), this finding is not certain and other possible causes, such as immune system damage due to toxic pollutants, are still being investigated (Refs. 12 through 16).

As emphasized by the ITC, PBDPE, DBDPE, and BTBPE have been detected in the environment (Ref. 1). PBDPE and DBDPE were found in air, soil, and sediments, and BTBPE was found in air and soil near two U.S. production facilities (Ref. 1). PBDPE has also been detected in dolphins found dead along the U.S. East Coast (Refs. 1 and 3). The ITC has cited several foreign references detailing PBDPE's presence in fish, marine mammals, and birds in Sweden, and in mussels and river sediment in Japan (Refs. 1). DBDPE was also detected in shellfish and sediments in Japan (Ref. 1).

DBDPE is on the list of toxic chemicals for the Toxics Release Inventory (TRI) established under section 313 of the Emergency Planning and Community Right-to-Know Act (Pub. L. 99-499, "EPCRA"). Facilities that manufacture, process or use DBDPE are required to annually report their DBDPE environmental releases to EPA. For the 1987 reporting year, the reported releases were over 155,000 pounds to air, over 20,000 pounds to water, and over 16,000 pounds to land (Ref. 1). While the other BFRs in this proposal are not on the TRI list, similar releases (adjusted for production volume) are likely because of their similarities in structure, manufacturing, processing, and use (Ref. 1).

Considering this evidence, EPA finds that the manufacturing, processing, distribution, use and/or disposal of these BFRs may pose an unreasonable risk of injury to health or to the environment due to developmental, chronic, oncogenic, or immunosuppressant effects. EPA also finds, based on information provided to EPA by the ITC and data EPA possesses, that for all of the proposed testing, insufficient data exist about the health or environmental effects of these BFRs to reasonably determine or predict the impacts of their manufacture, processing, distribution, use and/or disposal; and that testing is needed to develop such data (Refs. 1, 3, and 5).

IV. Proposed Rule and Test Standards

EPA is proposing that health and environmental effects and chemical fate testing be conducted on the BFRs in accordance with specific test guidelines set forth in Title 40 of the Code of Federal Regulations (CFR) as enumerated below in this document, except that the earthworm toxicity, chironomid toxicity, and revised combined chronic toxicity/oncogenicity test guidelines are proposed as written in this notice, and the immunotoxicity and biodegradation in water/sediment test guidelines are incorporated by reference in this notice.

A. Proposed Health Effects Testing and Test Standards

1. *Subchronic and chronic effects.* EPA is proposing subchronic toxicity testing for HBCD as specified in 40 CFR 798.2650.

2. *Neurotoxicity.* EPA is proposing neurotoxicity testing, including neuropathology, motor activity, and a functional observational battery for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 798.6400, 798.6200, and 798.6050.

3. *Reproductive effects.* EPA is proposing reproductive effects testing for PBDPE, OBDPE, BTBPE, and HBCD as specified in 40 CFR 798.4700. EPA finds the existing reproductive effects data available for DBDPE are inadequate (EPA believes that a 2-generation study is necessary for adequacy) and is therefore proposing reproductive effects testing for DBDPE, also. If this testing on PBDPE and DBDPE, which EPA is proposing to be performed prior to testing OBDPE, indicates to EPA that lack of reproductive effects cannot be reasonably predicted for OBDPE (i.e., if either PBDPE or DBDPE elicit reproductive effects), then EPA would require the initiation of testing on OBDPE by certified letter to the test sponsor(s).

4. *Developmental toxicity.* EPA is proposing developmental toxicity testing for PBDPE, as specified in 40 CFR 798.4900, in two mammalian species, a rat and a non-rodent. EPA is also proposing developmental effects studies in two species for OBDPE and HBCD. A developmental effects study in rats submitted to EPA for HBCD is inadequate due to incomplete reporting and to too few animals sampled in the study. A study in rats submitted for OBDPE is also not considered adequate by EPA because of too few animals used in the study (EPA requires 20 animals per dose group versus the 10 used). For DBDPE and BTBPE, EPA is proposing developmental effects testing in a non-rodent species only. Studies in rats submitted for DBDPE and BTBPE are adequate.

5. *Mutagenicity.* EPA is proposing tiered mutagenicity testing for each of the BFRs. For PBDPE, OBDPE and BTBPE, the available (negative) *Salmonella*/Ames data are adequate. For HBCD, the available *Salmonella* data (weakly positive) are also adequate. However, the *Salmonella* data on OBDPE are inconclusive, and, therefore, inadequate. Consequently, EPA is proposing *Salmonella* testing as specified in 40 CFR 798.5265 for OBDPE.

EPA is also proposing an *in vitro* gene mutation assay for PBDPE, OBDPE, BTBPE, and HBCD as specified in 40 CFR 798.5300. Available data on DBDPE are adequate for this effect. EPA is further proposing an *in vivo* cytogenetic assay, either as specified in 40 CFR 798.5385 (bone marrow aberrations) or 40 CFR 798.5395 (bone marrow micronucleus) for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD. An available *in vivo* bone marrow assay, presented no data in support of the conclusion that DBDPE does not induce chromosomal aberrations (Ref. 17). Therefore, EPA considers this study inadequate to address the concern for *in vivo* gene mutation effects for DBDPE.

EPA is also proposing that, for any of these substances, if either the proposed *Salmonella* or *in vitro* gene mutation testing yields positive mutagenicity results, then a sex-linked recessive lethal (SLRL) test in *Drosophila melanogaster* shall be conducted for that substance in accordance with 40 CFR 798.5275. If the SLRL test in *Drosophila melanogaster* is positive for any of these substances, then either a mouse visible or mouse biochemical specific locus test (MVSL or MBSL) shall also be conducted for that substance as specified in 40 CFR 798.5200 (MVSL) or 40 CFR 798.5195 (MBSL).

EPA is further proposing that, for any of these substances, if the proposed *in vivo* cytogenetics assay yields positive results, then a dominant lethal assay shall be conducted for that substance as specified in 40 CFR 798.5450. If the

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dominant lethal assay is positive for any of these substances then a heritable translocation assay would also be conducted for that substance in accordance with 40 CFR 798.5460.

6. *Oncogenicity and chronic toxicity.* EPA is proposing oncogenicity testing for PBDPE, OBDPE, BTBPE in mice as specified in 40 CFR 798.3300; EPA is further proposing that chronic effects and oncogenicity testing in rats be combined as specified in 40 CFR 798.3320 (which is modified in this proposed rule).

For HBCD, EPA is proposing oncogenicity testing in both rats and mice as specified in 40 CFR 798.3300 if positive mutagenicity results are obtained in either the gene mutation cells in culture assay, the sex-linked recessive lethal assay in *Drosophila melanogaster*, or the *in vivo* cytogenetics assay.

B. Proposed Environmental Effects Testing and Test Standards

For the following proposed environmental effects testing of the three diphenyl ethers, EPA is proposing that testing first be conducted on PBDPE. Only if testing on PBDPE yields a specified effect, would testing of OBDPE and DBDPE be required. EPA believes a tiered approach to testing the three diphenyl ethers for environmental effects is reasonable, given their similar structures and the possibility of limited toxicity or bioconcentration being expressed because of the large molecular size and low water solubility of these BFRs (Ref. 1). The alkyl phthalates consent order (54 FR 618, January 9, 1989) employed a similar approach.

EPA does not believe it can apply the results for PBDPE to BTBPE or HBCD and therefore testing of BTBPE and HBCD would proceed independently of the PBDPE testing.

EPA also believes, given the anticipated chemical and physical properties of the BFRs, that before testing for environmental effects begins, basic chemical fate data are needed on water solubility, vapor pressure, and degradation rates. The results of the chemical fate tests would identify upper levels for aquatic test concentrations and indicate potential testing problems. EPA is therefore proposing that this testing, with a reporting deadline of 6 months, be performed prior to initiating the environmental effects testing.

1. *Algal testing.* EPA is proposing that an algal assay be conducted for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 797.1050. Data on HBCD are inadequate for this effect due to questionable test substance purity. The analytical standard for measuring treatment concentrations was also not reported. Testing for OBDPE and DBDPE would be conditioned on obtaining from the algal testing of PBDPE an EC50 of ≦ 10 μg/L.

2. *Fish chronic toxicity.* EPA is proposing that chronic toxicity to fish be evaluated by conducting fish early life stage toxicity testing for rainbow trout and sheepshead minnow for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 797.1600. Testing for OBDPE and DBDPE for both fish species would be conditioned on obtaining from the early life stage testing of PBDPE a geometric mean maximum acceptable toxicant concentration (MATC) value of ≦ 10 μg/L.

3. *Invertebrate chronic toxicity.* EPA is proposing that aquatic invertebrate toxicity testing be conducted for PBDPE, OEDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 797.1330, for *daphnids*, and 40 CFR 797.1950, for mysid shrimp. Tests for OBDPE and DBDPE, for both organisms, would be conditioned on obtaining from either of the invertebrate chronic tests of PBDPE a geometric mean MATC value of ≦ 10 μg/L.

4. *Benthic organism toxicity.* EPA believes that, because of the expected tendency of these BFRs to partition into aquatic sediments, chronic testing on benthic organisms should be conducted with the midge (*Chironomus tetans* or *C. riparius*) for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as proposed in a new 40 CFR 795.135. Testing for OBDPE and DBDPE would be conditioned on obtaining from the benthic organism testing a geometric mean MATC value of ≦ 100 mg PBDPE/kg dry weight of sediment.

5. *Terrestrial organism toxicity.* EPA is proposing that toxicity to terrestrial organisms be evaluated for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD by conducting mallard reproduction testing as specified in 40 CFR 797.2150, and earthworm toxicity testing as proposed in a new 40 CFR 795.150. Mallard reproduction testing for OBDPE and DBDPE would be conditioned on obtaining from the mallard testing of PBDPE a NOEL of ≦ 500 ppm; and earthworm toxicity testing for OBDPE and DBDPE would be conditioned on obtaining from the earthworm testing of PBDPE an EC50 of ≦ 100 mg PBDPE/kg dry weight of soil.

6. *Terrestrial plant toxicity.* EPA is proposing that toxicity to terrestrial plants be evaluated for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD by conducting seed germination/root elongation toxicity testing as specified in 40 CFR 797.2750 and early seedling growth toxicity testing as specified in 40 CFR 797.2800. Both tests for OBDPE and DBDPE would be conditioned on obtaining an EC50 of ≦ 100 mg PBDPE/kg dry weight of soil in either test.

7. *Immunotoxicity.* EPA is proposing that immunotoxicity testing be conducted for PEDPE, OBDPE, DBDPE, BTBPE, and HBCD using the Jerne Plaque Assay, which is proposed to be incorporated by reference. Immunotoxicity testing for OBDPE and DBDPE would be conditioned on obtaining from the immunotoxicity testing of PBDPE a NOEL of ≦ 500 ppm.

8. *Bioconcentration.* EPA is proposing that bioconcentration testing be conducted in an acceptable fish species (fathead minnow, *Pimephales promelas*) for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 797.1520 (but modified to extend the exposure period to 91 days). Bioconcentration testing for OBDPE, and DBDPE would be conditioned on obtaining a bioconcentration factor of ≧ 1,000 with PBDPE.

C. Proposed Chemical Fate Testing and Test Standards

1. *Water solubility.* EPA is proposing that water solubility be determined using the generator column method for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 796.1860. EPA is also proposing that BFRs, which may have a water solubility of 10 ppb or less, be analyzed utilizing an electron-capture detector. Since an accurate measurement technique for the BFRs is available, these water solubilities shall be determined and reported, even if they are less than 10 ppb. Although EPA has water solubility figures for these substances, they are only estimates in the case of PBDPE, and were determined by inappropriate methodology in the case of OBDPE and DBDPE. Although the ITC did not recommend water solubility testing for BTBPE and HBCD, EPA believes a more rigorous procedure is warranted for these low water soluble compounds. Specifically, EPA is proposing that water solubility be determined not only in pure water, but also in dilution water. This is because water solubility as it is normally determined (in distilled water) may differ from what is obtained in the (dilution) water used for the aquatic toxicity tests. An accurate determination in dilution water at the salinity and temperature to be used in the toxicity tests is necessary to select the maximum concentration of test chemical in these tests.

2. *Octanol/water partitioning.* EPA is proposing that octanol/water partition

coefficients (K_{ow} values) be determined using the generator column method for PBDPE, OBDPE, and DBDPE, as specified in 40 CFR 796.1720. EPA finds present K_{ow} values inadequate because of inappropriate test methodologies.

3. *Vapor pressure.* EPA is proposing that vapor pressure be determined for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 796.1950.

4. *Sediment and soil adsorption.* EPA is proposing that sediment and soil adsorption testing be conducted for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 796.2750.

5. *Direct and indirect photolysis.* EPA is proposing that direct and indirect photolysis testing be conducted on the pure compounds; i.e., congenerically pure PBDPE, OBDPE, DBDPE, BTBPE and HBCD (see Unit IV.D. of this preamble), as specified in 40 CFR 796.3780, 796.3800 and 796.3700.

6. *Aerobic biodegradation in water/sediment.* EPA is proposing that aerobic biodegradation in water/sediment be conducted for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD using the ecocore system described by A.W. Bourquin, which is proposed to be incorporated by reference. EPA has examined the method described in A.W. Bourquin and has developed a sample matrix, available in the public record, for conducting preliminary and definitive core-chamber biodegradation tests using this method (Ref. 18). Testing for OBDPE and DBDPE would be conditioned on obtaining mineralization to CO_2 greater than 10 percent for PBDPE.

7. *Anaerobic biodegradation.* EPA is proposing that anaerobic biodegradation testing be conducted for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in 40 CFR 796.3140. The ITC noted, and EPA anticipates, that these substances may undergo reductive debromination. Therefore, this anaerobic biodegradation testing on OBDPE and DBDPE is not conditioned on the results for PBDPE.

D. Test Substances

EPA is proposing testing of PBDPE, OBDPE, DBDPE, BTBPE, and HBCD of at least 98 percent purity as the test substances. EPA recognizes that the three diphenyl ethers are not pure congeneric forms, with each having a single level of bromination (i.e., purely "penta," "octa" or "deca" brominated forms). EPA also recognizes that they are not pure isomers (i.e., brominated not only at a specific level, but also at specific positions on the diphenyl ether molecule). Instead, they are a complex composition of diphenyl ether compounds brominated to different degrees and at different positions (Ref. 16). For example, PBDPE is composed of primarily tetra-, penta-, and hexabrominated diphenyl ethers but with even higher and lower brominated forms present in commercial PBDPE.

EPA is proposing that the test substance reflect the composition of the commercial substance in terms of the mix of the individual brominated congeners present in the commercial substance. The purity specifications proposed above pertain to reducing the amount of chemicals other than brominated diphenyl ethers present in the test substance. EPA is further proposing that the test substance being used in each test be analyzed to determine the percent composition of the different brominated congeners present.

EPA has specified relatively pure substances for testing because EPA is interested in evaluating the effects attributed to the subject substances themselves. This increases the likelihood that any toxic effects observed are related to the subject BFRs and not to any impurities. Potential test sponsors for the three diphenyl ethers and for BTBPE should also be aware of EPA's concern that these BFRs may be contaminated with halogenated dibenzodioxins (HDDs)/dibenzofurans (HDFs) as set forth in 40 CFR part 766. Given the known toxicity of these impurities, test sponsors should take special care to eliminate or minimize any possible contamination with HDDs/HDFs, where they believe these contaminants may be present.

EPA solicits comments on the test substance composition. (see Unit V of this preamble).

E. Persons Required to Test

Because of the findings in Unit III of this preamble, EPA is proposing that persons who manufacture (including import) and/or process, or who intend to manufacture and/or process PBDPE, OBDPE, DBDPE, BTBPE and/or HBCD, other than as an impurity, at any time from the effective date of the final test rule to the end of the reimbursement period, be subject to the testing requirements. Byproduct manufacturers and importers of PBDPE, OBDPE, DBDPE, BTBPE, and/or HBCD are considered manufacturers under this rule. As explained in 40 CFR part 790, manufacturers but not small quantity manufacturers, processors, or research and development manufacturers of PBDPE, OBDPE, DBDPE, BTBPE, and/or HBCD would be required to submit letters of intent or exemption applications.

EPA has specified relatively pure substances for testing. EPA would not require submission of equivalence data as a condition for exemption from testing since EPA is interested in evaluating the effects attributable to PBDPE, OBDPE, DBDPE, BTBPE, and/or HBCD.

F. Reporting Requirements

Data developed under the final rule would be reported in accordance with TSCA Good Laboratory Practice (GLP) Standards, 40 CFR part 792.

As required by section 4(b)(1)(C) of TSCA, EPA is proposing specific reporting requirements for each of the proposed tests for PBDPE, OBDPE, DBDPE, BTBPE, and HBCD as specified in the following Table 1.

TABLE 1.—PROPOSED HEALTH AND ENVIRONMENTAL EFFECTS AND CHEMICAL FATE TESTING AND REPORTING REQUIREMENTS FOR THE BFRS

Test Standard in 40 CFR	Test substances	Reporting deadline for final report(months)[1]	Number interim(6 month) reports required[1]
A. Health Effects:			
Subchronic toxicity[2] (§ 798.2650)	HBCD	18	2
Combined chronic toxicity/oncogenicity (§ 798.3320)	PBDPE, OBDPE, BTBPE	53	8
Oncogenicity (§ 798.3300)	PBDPE, OBDPE, BTBPE, HBCD	53	8

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TABLE 1.—PROPOSED HEALTH AND ENVIRONMENTAL EFFECTS AND CHEMICAL FATE TESTING AND REPORTING REQUIREMENTS FOR THE BFRS—Continued

Test Standard in 40 CFR	Test substances	Reporting deadline for final report(months)[1]	Number interim(6 month) reports required[1]
Neurotoxicity (§ 798.6065, 798.6200, 798.6400)	PBDPE, OBDPE, DBDPE, BTBPE,HBCD	21	3
Reproductive toxicity[2] (§ 798.4700)	PBDPE, OBDPE, BTBPE, HBCD, DBDPE	29	4
Developmental toxicity (§ 798.4900)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	12	1
Salmonella assay (§ 798.5265)	OBDPE	9	1
In vitro gene mutation assay (§ 798.5300)	PBDPE, OBDPE, BTBPE, HBCD	10	1
In vivo cytogenetics assay (§ 798.5385 or 798.5395)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	14	2
Drosophila sex-linked recessive lethal test[2] (§ 798.5275)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	22	1
Mouse specific locus test[2] (§ 798.5200 or 798.5195)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	51	8
Rodent dominant lethal test[2] (§ 798.5450)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	36	3
Heritable translocation test[2] (§ 798.5460)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	25	4
B. Environmental Effects:			
Algal test[2] (§ 797.1050)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	15 [3]24	1 1
Rainbow trout life stage test[2] (§ 797.1600)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	18 [3]30	1 1
Sheepshead minnow life stage test[2] (§ 797.1600)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	18 [3]30	1 1
Daphnid chronic test[2] (§ 797.1330)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	18 [3]30	1 1
Mysid shrimp chronic test[2] (§ 797.1950)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	15 [3]24	1 1
Chironomid sediment toxicity test[2] (§ 795.135)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	18 [3]30	1 1
Mallard reproduction test[2] (§ 797.2150)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	18 [3]30	1 1
Earthworm toxicity test[2] (§ 795.150)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	18 [3]30	1 1
Seed germination/root elongation test[2] (§ 797.2750)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	15 [3]24	1 1
Early seedling growth test[2] (§ 797.2800)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	15 [3]24	1 1
Immunotoxicity test[2]	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	15 [3]24	1 1
Bioconcentration[2] (§ 797.1520)	PBDPE, OBDPE, DBDPE, BTBPE, HBCE	18 [3]30	1 1
C. Chemical Fate:			
Water solubility (§ 796.1860)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	6	0
Log octanol/water partition testing (§ 796.1720)	PBDPE, OBDPE, DBDPE	6	0
Vapor pressure testing (§ 796.1950)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	6	0
Sediment and soil adsorption testing (§ 796.2750)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	6	0
Direct and indirect photolysis testing (§ 796.3780, 796.3800, 796.3700)	PBDPE, OBDPE, DBDPE, BTBPE,HBCD	6	0
Biodegradation testing in water/sediment[2]	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	12 [3]24	1 1
Anaerobic biodegradation testing (§ 796.3140)	PBDPE, OBDPE, DBDPE, BTBPE, HBCD	6	0

[1] Figure indicates the reporting deadline in months calculated from the effective date of the final rule or from the date of test sponsor notification by certified letter to initiate test where such notification is specified.
[2] For one or more of these test substances, this test requirement is conditional as described in Unit IV of this preamble.
[3] This figure is the reporting deadline for testing on OBDPE and DBDPE, which is conditioned on results from testing PBDPE.

V. Issues for Comment

EPA welcomes comment on this proposed testing. In particular, EPA solicits comment in the following four areas:

- Test substance composition.
- Categorization for testing purposes.
- New test guidelines.
- Route of test substance administration.

A. Test Substance Composition

EPA is proposing that the test substance used in the testing should represent, with some purity specifications, what is actually manufactured, rather than attempting to

test a single congeneric substance (i.e., a "pure but representative composition" of 4-, 5-, and 6-brominated compounds, which typifies commercial PBDPE is proposed to be tested instead of a composition confined to pentabrominated isomers). On the other hand, EPA also believes that it could be beneficial to use a congenerically pure substance to reduce the number of potentially confounding variables in any future hazard or risk assessment activities for these substances. However, EPA recognizes the potential difficulty in isolating congenerically pure substances of this type, and EPA is also concerned that if congenerically pure substances were used then these test substances would not fairly represent what humans are actually exposed to or what is actually released to the environment (i.e., the commercial mixture). For these reasons, EPA is proposing to require testing of representative test substances composed of differing congeners, similar to what is sold commercially. Nonetheless, EPA is also considering that testing for any or all of the proposed tests may be of congenerically pure substances, if comments and/or data received prior to promulgation convince EPA that these would provide the most useful or interpretable data. For example, EPA is proposing that congenerically pure substances be used in the direct and indirect photolysis testing. EPA believes that the technical limitations inherent in this testing require that congenerically pure substances be used to obtain useful results.

For meaningful interpretation of results, EPA needs to know what is present in the test substance, and EPA is therefore proposing that the test substances be analyzed to determine overall purity and the percentage of each congener present in the mixture. Further, EPA is proposing that, when tested as a commercially representative substance, PBDPE contain not less than 58 percent pentabrominated diphenyl ethers, that OBDPE contain not less than 30 percent octabrominated diphenyl ethers, and that DBDPE contain not less than 98 percent decabrominated diphenyl ethers, and also that BTBPE should contain not less than 98 percent pure 1,2-bis(2,4,6-tribromophenoxy)ethane, and HBCD should contain not less than 98 percent pure hexabromocyclododecane.

For the photolysis tests and any others in which PBDPE, OBDPE, and DBDPE are tested as congenerically pure substances, EPA is proposing that the respective penta-, octa-, and decabrominated isomers make up not less than 90 percent of these substances. EPA is specifically soliciting comments on testing these BFRs in forms representative of what is produced commercially or, instead, as pure congeneric forms; and also on the proposed purity standards and chemical analyses.

B. Chemical Categorization

EPA has concluded that the three diphenyl ethers, PBDPE, OBDPE, and DBDPE, are similar enough in structure to be considered a single category for certain testing purposes. For example, EPA is proposing that reproductive effects testing for OBDPE be conditioned on receiving a positive response with PBDPE or DBDPE. EPA is also proposing to condition the environmental effects testing and one chemical fate test for OBDPE and DBDPE on the results obtained from PBDPE.

EPA is soliciting comment on the scientific appropriateness of conditioning testing in this way for these BFR substances, and also on whether the categorization proposed in this notice is too limited or too broad in the context of each test.

C. Test Guidelines

EPA is proposing three guidelines which are either new or modified, and is also proposing methodologies for two additional tests, which are incorporated by reference.

Both the Chironomid Test and the Earthworm Toxicity Test standards are based on new EPA test guidelines. EPA believes that these tests are necessary, and will help to evaluate the potential risk to benthic aquatic and terrestrial organisms, respectively, from exposure to the BFRs.

The Combined Oncogenicity/Chronic Toxicity guideline, 40 CFR 798.3320, has never been promulgated as a test standard in any test rule. EPA is proposing that certain aspects of the Combined Oncogenicity/Chronic Toxicity guideline be made "shall" rather than "should" testing requirements to make these parts of the guideline enforceable. EPA considers a "shall" requirement to be an essential aspect of the test methodology. Violation of a "shall" requirement is considered a serious breach of test performance and may result in penalties and/or non-acceptance of the test results by EPA.

A fourth test, the Jerne Plaque Assay, is being proposed for BFRs. The Jerne Plaque Assay would be incorporated by reference in the final test rule. The Jerne Plaque Assay evaluates immunotoxicity in mice and is a standard test for this effect. Although this test is a surrogate human health effects test, EPA believes that this test is also adequate for evaluating the risk of possible immunotoxicity effects in environmental species, especially mammals.

Finally, EPA is proposing an aerobic biodegradation test in water/sediment (also known as the ecocore test system) using the methodology of A.W. Bourquin, which would be incorporated by reference in the final test rule. EPA has required the ecocore test system in previous EPA test rules (e.g., in the final test rule for tetrabromobisphenol A, 52 FR 25219, July 6, 1987).

EPA solicits comment on these five protocols.

D. Route of Test Substance Administration

Although a major route of exposure of humans to the BFRs is by inhalation, it is difficult in toxicity testing to maintain consistent, reliable exposures of powdery solid test substances like the BFRs using the inhalation route of administration. Therefore, EPA is proposing that the health effects testing be conducted by the oral route. Specifically, EPA is proposing testing by gavage, because it believes that this route will provide a consistent, reliable dose and reliable results. EPA does not believe, in this case, that the toxicology of the BFRs using the oral route will be significantly different from the inhalation route. Previous testing done on the BFRs by the oral route has shown effects on the liver consistent with those of a 14-day inhalation study. Furthermore, the only positive oncogenicity assay (with DBDPE) was also performed using an oral (dietary) route of administration. However, EPA is soliciting comments on this issue, and if comments indicate that the inhalation (or other route) should be used, in any or all testing, then EPA may require that route of administration.

VI. Economic Analysis of the Proposed Rule

EPA prepared an economic analysis that evaluates the potential for significant economic impacts as a result of the proposed testing. (Ref. 2). Total testing costs are estimated to range from $11.6 to $19.1 million. These costs have been annualized and compared with annual revenue as an indication of potential impact. These annualized costs represent equivalent constant costs which would have to be recouped each year of the payback period to finance the testing expenditure in the first year.

The annualized test costs, using a 7 percent cost of capital over a period of

15 years, are as follows: DBDPE — $102,000 to $154,000; HBCD — $254,000 to $427,000; BPDPE and BTBPE -$309,000 to 504,000; and OBDPE — $308,000 to 505,000. The production volume and price information have been claimed confidential and are contained in the economic analysis, which is being treated as CBI.

VII. Availability of Test Facilities and Personnel

EPA has determined that test facilities and personnel are available to perform the testing specified in this proposed rule. (Ref. 19).

VIII. Public Meeting

If requests for oral comments are submitted, EPA will hold a public meeting in Washington, DC after the close of the public comment period. Persons who wish to attend or to present comments at the meeting should call Mary Louise Hewlett, Chemical Testing Branch (202) 475–8162 by August 9, 1991. The meeting will be open to the public, but active participation will be limited to those who requested to comment and EPA representatives. Participants are requested to submit copies of their statements by the meeting date. These statements and a transcript of the meeting will become part of EPA's rulemaking record.

IX. Comments Containing Confidential Business Information

All comments will be placed in the public file unless they are clearly labeled as Confidential Business Information (CBI) when they are submitted. While a part of the record, CBI comments will be treated in accordance with 40 CFR part 2. A sanitized version of all CBI comments should be submitted, if possible, to EPA for the public file.

It is the responsibility of the commenter to comply with 40 CFR part 2 in order that all materials claimed as confidential may be properly protected. This includes, but is not limited to, clearly indicating on the face of the comment (as well as on any associated correspondence) that CBI is included, and marking "CONFIDENTIAL", "TSCA CBI" or similar designation on the face of each document or attachment in the comment which contains CBI. Should information be put into the public file because of failure to clearly designate its confidential status on the face of the comment, EPA will presume any such information which has been in the public file for more than 30 days to be in the public domain.

X. Rulemaking Record

EPA has established a record for this rulemaking (docket number OPTS–42115). This record contains the basic information considered by EPA in developing this proposal and appropriate Federal Register notices. EPA will supplement this record as necessary.

A public version of the record, from which all CBI has been deleted, is available for inspection in the TSCA Public Reading Room, G–004, NE Mall, 401 M St., SW., Washington, DC 20460, from 8 a.m. to 12 noon, and 1 p.m. to 4 p.m., Monday through Friday, except legal holidays. The record includes the following information:

A. Supporting Documentation

(1) Notice containing the ITC designation.

(2) Federal Register notices pertaining to this rule consisting of:

(a) Notice of final rule on EPA's TSCA Good Laboratory Practice Standards (54 FR 34034; August 17, 1989).

(b) Notice of final rule on data reimbursement policy and procedures (48 FR 31786, July 11, 1983).

(3) TSCA test guidelines cited as test standards for this rule.

(4) Communications consisting of:

(a) Written letters.

(b) Contact reports of telephone conversations.

(c) Meeting summaries.

B. References

(1) USEPA. U.S. Environmental Protection Agency. "Twenty-fifth Report of the Interagency Testing Committee to the Administrator; receipt of report and request for comments regarding priority list of chemicals." (December 12, 1989, 54 FR 51114).

(2) Szarek, P. "Economic analysis of proposed test rule for five brominated flame retardants non-CBI version". Memorandum from Pat Szarek to John Schaeffer, USEPA, Office of Pesticides and Toxic Substances, Washington DC (October, 1990).

(3) USEPA. Environmental Research Laboratory, Duluth MN. "Brominated chemicals as marine contaminants." Memorandum from Steven J. Broderius to Maurice Zeeman, Washington, DC, Office of Toxic Substances, USEPA (February 14, 1990).

(4) MRI. Midwest Research Institute "Mass spectral confirmation of chlorinated and brominated diphenyl ethers in human adipose tissues." Final Report for USEPA, Exposure Evaluation Division, Office of Toxic Substances, EPA Contract No. 68–02–4252. (June, 1990).

(5) USEPA. "Brominated flame retardants–post-RM 1 meeting revision of HERD testing recommendations". Memorandum from Mark W. Townsend to Gary E. Timm, Washington, DC, Office of Pesticides and Toxic Substances, USEPA (June 16, 1990).

(6) NTP. National Toxicology Program. "Toxicology and carcinogenesis studies of decabromodiphenyl oxide (CAS No. 1163–19–5) in F344/N rats and B6C3F1 mice (feed studies)." NTP Technical Report Series No. 309, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (1986).

(7) Kociba, R.J., Frauson, L.O., Humiston, C.G., Norris, J.M., Wade, C.E., Lisowe, R.W., Quast, J.F., Jersey, G.C., and Jewett, G.L. "Results of a two–year dietary feeding study with decabromodiphenyl oxide (DBDPO) in rats." *Journal of Fire and Flammability/Combustion Toxicology*. 2:267–285 (1975).

(8) Norris, J.M., Ehrmantraut, J.W., Gibbons, C.L., Kociba, R.J., Schwetz, B.A., Rose, J.Q., Humiston, C.G., Jewett, G.L., Crummett, W.B., Gehring, P.J., Tirsell, J.B., and Brosier, J.S. "Toxicological and environmental factors involved in the selection of decabromodiphenyl oxide as a fire retardant chemical." *Journal of Fire and Flammability/Combustion Toxicology, Supplement*. 1:52–77 (1974).

(9) Walsh, G.E., Yoder, M.J., McLaughlin, L.L and Lores, E.M. "Responses of marine unicellular algae to brominated organic compounds in six growth media." *Ecotoxicology and Environmental Safety*. 14:215–222 (1987).

(10) Wong, K.F. "Production/exposure profile for brominated diphenyl oxide." USEPA, Office of Toxic Substances, Chemical Engineering Branch (January 7, 1986).

(11) USEPA. "Twenty-fifth ITC report comments on the oncogenicity testing recommendations for five brominated flame retardants." Memorandum from Ann Clevenger to Carol A. Bellizzi, Washington, DC, Office of Pesticides and Toxic Substances, USEPA (February 2, 1990).

(12) Times Wire Service. "Dolphin deaths traced to 'red tide'." *Los Angeles Times*, Section 1, page 2 (February 1, 1989).

(13) Hilts, P.J., and Leff, L. "Toxic algae killed dolphins; marine mammal catastrophe blamed on poisonous 'red tide'." *The Washington Post*, Metro section, page D O1 (February 2, 1989).

(14) Jones, J.L. "Navy asked to help ill dolphins", *Los Angeles Times*, Orange County Edition, Metro section, page 3 (November 17, 1989).

(15) Lancaster, J. "New surge in dolphin deaths triggers probe; more than 300 bottlenoses have washed ashore from Gulf of Mexico since January." *The Washington Post*, Section A, page A 21 (May 11, 1990).

(16) Carlson, G.P. "Induction of xenobiotic metabolism in rats by short-term administration of brominated diphenyl ethers." *Toxicology Letters*. 5:19–25 (1980).

(17) Norris, J.M., Kociba, R.J., Schwetz, B.A., Rose, J.Q., Humiston, C.G., Jewett, G.L., Gehring, P.J., and Mailhes, J.B. "Toxicology of Octabromobiphenyl and Decabromodiphenyl Oxide." *Environmental Health Perspectives*. 11:153–161 (1975).

(18) USEPA. "Matrix for conducting preliminary and definitive core-chamber biodegradation tests." Draft paper by John D. Walker, Washington, DC, Office of Toxic Substances, USEPA (May 11, 1988).

(19) Booz, Allen, Hamilton., Inc., Bethesda, MD. "EPA census of the toxicological testing industry." Prepared for the Office of Policy

Analysis OTS, USEPA, Washington, DC (June 1990).

XI. Othe Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA must judge whether a rule is "major" and therefore subject to the requirement of a Regulatory Impact Analysis. EPA has determined that if promulgated, this proposed test rule would not be major because it does not meet any of the criteria set forth in section 1(b) of the Order; i.e., it would not have an annual effect on the economy of at least $100 million, would not cause a major increase in prices, and would not have a significant adverse effect on competition or the ability of U. S. enterprises to compete with foreign enterprises.

This proposed rule was submitted to the Office of Management and Budget (OMB) for review as required by Executive Order 12291. Any written comments from OMB to EPA, and any EPA response to those comments, are included in the rulemaking record.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (5 U.S.C. 601 et seq., Pub. L. 96–354, September 19, 1980), EPA is certifying that this test rule, if promulgated, would not have a significant impact on a substantial number of small businesses because: (1) They would not be expected to perform testing themselves, or to participate in the organization of the testing effort; (2) they would experience only very minor costs, if any, in securing exemption from testing requirements; and (3) they are unlikely to be affected by reimbursement requirements.

C. Paperwork Reduction Act

OMB has approved the information collection requirements contained in this proposed rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 et seq., and has assigned OMB Control number 2070–0033.

Public reporting burden for this collection of information is estimated to average 68,800 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. The total public reporting burden is estimated to be 206,400 hours for all responses.

Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM–223, U.S. Environmental Protection Agency, 401 M St., SW., Washington, DC 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070–0033), Washington DC 20503. The final rule will respond to any OMB or public comments on the information collection requirements contained in this proposal.

List of Subjects in 40 CFR Parts 795, 798 and 799

Chemicals, Chemical export, Chemical fate, Environmental effects, Environmental protection, Hazardous substances, Health effects, Incorporation by reference, Laboratories, Reporting and recordkeeping requirements, Testing.

Dated: June 17, 1991.

Victor J. Kimm,

Acting Assistant Administrator for Pesticides and Toxic Substances.

Therefore, it is proposed that 40 CFR chapter I, subchapter R, be amended as follows:

1. In part 795

PART 795 — [AMENDED]

a. By revising the authority citation for part 795 to read as follows:

Authority: 15 U.S.C. 2601, 2603

b. By adding § 795.135 to read as follows:

§ 795.135 Chironomid sediment toxicity test.

(a) *Purpose.* This guideline may be used to develop data on the toxicity and bioavailability of chemical substances and mixtures ("chemicals") in sediments subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469,90 Stat. 2003, 15 U.S.C. 2601 et. seq.). This guideline prescribes tests to be used to develop data on the toxicity of chemicals present in sediments to chironomid larvae (midges). The U.S. Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of TSCA and 40 CFR part 792, Good Laboratory Practice Standards (GLPS), apply to this test guideline. In addition, the following definitions also apply:

Bioconcentration Factor (BCF) is the quotient of the concentration of a test substance in tissues of the chironomids at or over a specific time period of exposure divided by the concentration of test substance in the overlying water, interstitial water, or in the sediments at or during the same time period.

Cation exchange capacity (CEC) means the sum total of exchangable cations that a sediment can absorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meg/100g) or milliequivalents of negative charge per gram (meq/g) of sediment (dry weight).

EC50 means an experimentally-derived concentration of test substance in the sediment that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time.

Flow-through means a continuous or intermittent passage of dilution water through a test chamber or culture tank with no recycling of water.

Geometric mean MATC is the calculated mean between the highest test concentration with no statistically significant effects and the lowest concentration showing significant effects.

Interstitial water is liquid which is found in or directly adjacent to sediments and can be extracted from these sediments by several processes.

Loading means the ratio of chironomid biomass (grams wet weight) to the volume (liters) of test solution in a test chamber at a point in time or passing through the test chamber during a specific interval.

Lowest observed effect concentration (LOEC) means the lowest treatment (i.e., test concentration) of a test substance that is statistically different in adverse effect on a specific population of test organisms from that observed in controls.

MATC (Maximum Acceptable Toxicant Concentration) means the maximum concentration at which a chemical may be present and not be toxic to the test organism.

No observed effect concentration (NOEC) means the highest treatment (i.e., test concentration) of a test substance that shows no statistical difference in adverse effect on a specific population of test organisms from that observed in controls.

Overlying water is liquid which is found above or placed over sediments. For purposes of this guideline, overlying water is equivalent to the term "water column".

Partial life-cycle toxicity test is one which uses a sensitive portion of the life of a test organism (second instar of midges) to assess the effects of test substances.

Redox potential (E_h) means the oxidizing or reducing intensity or condition of a solution expressed as a current, referenced against a hydrogen electrode. Within wet sediments

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reducing conditions prevail such that zero or negative E_h values may be present.

...iment is matter which settles to the ...ottom of a liquid in natural ...tuations or a substrate prepared from ...combination of natural sediments and ...rtificial components. "Sediment" is equivalent to the term "solid-phase sediments" in this guideline.

Sediment partition coefficient is the ratio of the concentration of test substance on the sediment to the concentration in the overlying water. For the purposes of this guideline, this term is identical to "soil-water partition coefficient."

Spiking is the addition of a test substance to a negative control and/or reference sediment so that the toxicity of a known quantity of test substance can be determined in a known nontoxic sediment. Often a solvent carrier is needed for low-water soluble test substances.

Subchronic toxicity test means a method used to determine the concentration of a test substance in water and for sediment which produces an adverse effect on chironomids over a partially extended period of time. In this guideline, mortality and growth (expressed as change in wet weight of midges) are the criteria of toxicity.

(c) *Test procedures* — (1) *Summary of test.* (i) This flow-through test consists of three parts. First is a 14–day aqueous exposure test, with minimal sediments, with food, and with the test substance added to the overlying water. Second is a 14–day sediment exposure test, with one or more sediments (4 to 6 cm in thickness) which may have varying amounts of organic carbon, with food, and with the test substance added to sediment(s). Third is a 14–day interstitial exposure test, with one or more sediments (4 to 6 cm in thickness) which may have varying amounts of organic carbon, with food, and with the test substance added to overlying water. The flow-through test is illustrated in the following Table 1.

TABLE 1.—EXPERIMENTAL DESIGN FOR THE CHIRONOMID SEDIMENT FLOW-THROUGH TOXICITY TEST

Test system	Test substance concentrations (2 reps ea) [a]	Number of sediments (2 reps ea)	Number of Samples Analyzed (2 reps ea)			
			Overlying water P/C [b]	Interstitial water P/C [b]	Sediments	Midges [c]
1. 14–Day Aqueous Exposure	5(10)	NA[d]	5(10)	NA	NA	5(10)
Control (2 reps)	NA	NA	1(2)	NA	NA	1(2)
Solvent Control (2 reps)	NA	NA	1(2)	NA	NA	1(2)
2. 14–Day Sediment Exposure	5(10)	1-3 [e]	(2-6)	5(10)	NA	5(10)
Control (2 reps)	NA	1(2)	1(2)	NA	1(2)	1(2)
Solvent Control (2 reps)	NA	1(2)	1(2)	NA	1(2)	1(2)
3. 14–Day Interstitial Water/Sediment Exposure	5(10)	1-3[e]	(2-6)	5(10)	5(10)	5(10)
...ol (2 reps)	NA	1(2)	1(2)	1(2)	1(2)	1(2)
Solvent Control (2 reps)	NA	1(2)	1(2)	1(2)	1(2)	1(2)

[a] Test substance concentration in all replicates measured at days 0 and 14. Reps = replicates
[b] P/C = physical chemical measurements (dissolved oxygen, temperature (°C), and pH) on days 0, 4, 7, 10, and 14.
[c] Midges are observed throughout the test, dead chironomids recorded, removed and weighed on days 4, 7, and 10. At end of each test, remaining midges from each replicate are removed, counted, and weighed.
[d] NA = not applicable
[e] Number of sediment types tested will depend on range of TOC content tested; 1 to 3 types (low, medium, and high TOC levels) are recommended.

(ii) The day before the test is to be started, sediments (in treatments, and reference and negative controls) shall be screened to remove large particles and endemic animals (especially midge predators) added to the test chambers. The amount of sediments to be added to each test chamber will depend on the experimental design and test species. Only a minimum amount (2mm) shall be added in the aqueous exposure portion of the test. Each replicate test chamber should contain the same amount of sediments. Overlying water shall then be added to each test chamber.

(iii) In this flow–through test, the flow of dilution water through each chamber is begun and then adjusted to the rate desired. The test substance shall be introduced into each test chamber. The addition of test substance in the flow-through system shall be done at a rate which is sufficient to establish and ma...in the desired concentration of tes...ubstance in the test chamber.

(iv) At the initiation of the test, chironomids which have been cultured or acclimated in accordance with the test design, are randomly placed into the test chambers. Midges in the test chambers are observed periodically during the test. Immobile or dead larvae shall be counted, removed, and weighed, and the findings are recorded. "Floating" larvae are nonviable and shall be replaced. Dissolved oxygen (DO) concentration, pH, temperature, the concentration (measured) of test substance, and other water quality parameters are measured at specified intervals in selected test chambers, during all three parts of this test (See Table 1 in paragraph (c)(1)(i) of this section). Data shall be collected during the test to determine any significant differences ($P \leq 0.05$) in mortality and growth as compared to the controls. BCFs shall be calculated at the end of the test, based on route of exposure.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted prior to beginning each of the three parts of the test to establish test solution concentrations for the three definitive parts of the test.

(ii) The chironomids should be exposed to a series of widely spaced concentrations of the test substance (e.g., 1, 10, 100 mg/L).

(iii) A minimum of 10 chironomids should be exposed to each concentration of test substance for a period of time which allows estimation of appropriate test concentrations. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive portion of the test is to determine concentration-response curves, EC50 values, effects of a chemical on mortality and growth, and the determination of BCFs during subchronic exposure.

(ii) A minimum of 30 midges per concentration (15 midges per replicate test chamber) should be exposed, in each part of the test, to 5 or more concentrations of the test substance chosen in a geometric series in which the ratio is between 1.5 and 2.0 mg/L (e.g., 2, 4, 8, 16, 32, 64 mg/L). An equal number of chironomids should be placed in two replicates. The concentration ranges should be selected to determine the concentration-response curves, EC50 values, and MATC. Solutions should be analyzed for chemical concentration prior to use and at designated times during the test.

(iii) Each test shall include controls consisting of the same dilution water, sediments, conditions, procedures, and midges from the same population (same egg mass in culture container), except that none of the test substance is added.

(iv) The test duration is 14 days for each of the three parts of the test. The test is unacceptable if more than 20 percent of the control organisms are dead, stressed or diseased during the test. For high log K_{ow} chemicals, a test period longer than 14 days may be necessary.

(v) The number of dead chironomids in each test chamber shall be recorded on days 4, 7, 10, and 14 of the test. At the end of the test, surviving midges are removed from the test chambers and weighed after blotting dry. Concentration-response curves, EC50 values, and associated 95 percent confidence limits for mortality shall be determined for days 4, 7, 10, and 14 in the aqueous exposure portion of the test. Also, an MATC, as well as NOEC and LOEC values shall be determined for midge survival and growth.

(vi) In addition to survival and growth, any abnormal behavior or appearance of the chironomids should be reported.

(vii) Distribution of midges among the test chambers shall be randomized. In addition, test chambers within the testing area are positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(viii) A control sediment and/or a reference sediment shall be used in each part of this test. Use of these controls/references will help determine if the test is acceptable, serve to monitor the health of the chironomids used in the testing, monitor the quality and suitability of test conditions, parameters and procedures, and aid in analyzing data obtained from this test. A negative control shall be run in the test, and this is to be a sediment known to be non-toxic to the midges. Also, in addition to, or in place of the negative control, a reference sediment can be run in the test. The reference sediment is obtained from an area that is known to have low levels of chemical contamination and which is similar to or identical to the test sediments (in physical and chemical characteristics).

(ix) In the first part of this test, the aqueous exposure, a minimal amount of sediments (≦ 2mm) is placed in the test chambers. Sediments are necessary to reduce stress to the chironomids, cannibalism, and to allow the midges to construct tubes.

(x) BCFs shall be calculated at the end of each part of the test.

(5) [Reserved]

(6) *Analytical measurements* — (i) *Water quality analysis.* (A) The hardness, acidity, alkalinity, conductivity, total organic carbon (TOC) or chemical oxygen demand (COD), and particulate matter of the dilution water serving as the source of overlying water shall be measured on days 0 and 14. The month-to-month variation of these values should be less than 10 percent and the pH should vary less than 0.4 units.

(B) During all three parts of the flow-through test, DO, temperature, and pH shall be measured in each chamber on days 0, 4, 7, 10, and 14.

(ii) *Measurement of test substance.* (A) Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses of water and sediments. Radiolabeling of the test substance (e.g., by use of ^{14}C) may be necessary in order to accurately measure quantities present in the sediments. The analytical method used to measure the amount of test substance in the sample shall be validated by appropriate laboratory practices before beginning the test. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interference which cannot be systematically identified and corrected mathematically. When radiolabeled test substances are used, total radioactivity shall be measured in all samples. At the end of the test, water, sediments, and tissue samples should be analyzed using appropriate methodology to identify and estimate any major (at least 10 percent of the parent compound) degradation products or metabolites that may be present.

(B) For all three aqueous exposure parts of this test, the overlying water shall be sampled on days 0, 7, and 14 from each test chamber, for the test substance.

(C) For the non-aqueous exposure parts of the test, the interstitial water shall be sampled for the test substance on days 0, 7, and 14 from each test chamber. Interstitial water can be sampled by using a variety of methods, such as removal of overlying water and centrifugation, filtration of sediments, pressing the sediments, or using an interstitial water sampler. Care should be taken during these measurements to prevent the biodegradation, transformation, or volatilization of the test substance.

(D) For the non-aqueous exposure portion of the test, the sediments shall be sampled for the test substance on days 0, 7, and 14 from each test chamber.

(E) The sediment partition coefficient or soil-water partition coefficient is determined by dividing the average test substance sediment concentration by the respective average water column concentration. Concentrations of test substance in the sediments to be used in this test can be chosen by measuring these partition coefficients. This sediment partition coefficient should be determined in triplicate by placing a quantity of a sediment with a known TOC content spiked with the radiolabeled test substance into a quantity of dilution water. The ratio of sediment to dilution water should simulate the ratio present in the test. The sediment/dilution water mixture is periodically shaken, and the radiolabeled test substance is measured. This shaking and sampling procedure is repeated until equilibrium is reached, as defined by the stage of the desorption curve.

(F) Overlying water samples should be filtered through a 0.45 micron filter to determine the concentration of dissolved test substance.

(G) BCFs shall be calculated by determining the amount of test substance in the midge tissue divided by concentrations of test substance in the water column, interstitial water, and sediments. At test termination, the midges remaining in each test concentration are analyzed for test substance. Suitable methods are available, such as radiolabeling (^{14}C) the test substance, combusting the midges, and trapping and counting the resulting radioactivity, if other methods are unavailable. The BCF can then be calculated. If insufficient chironomid biomass is present at the conclusion of the test, then replicates may be pooled, if necessary. If this pooling still results in insufficient biomass or if the accumulated test substance concentration is lower than the

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detection limit for the test substance. BCFs cannot be calculated.

(iii) *Numerical.* (A) The number of d midge second instars shall be nted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for mortality concentration-response curves calculated on days 4, 7, 10, and 14. A 4–, 7–, 10–, and 14–day LC50 value based on second instar mortality, and with corresponding 95 percent confidence intervals, shall be calculated. The methods recommended for calculating EC50's include probit, logit, binomial, and moving average.

(B) Appropriate statistical tests (e.g., analysis of variance and mean separation tests) should be used to test for significant chemical effects on growth (measured as wet weights) on days 4, 7, and 14. An MATC shall be calculated using these test criteria.

(C) In no case should any analytical measurements be pooled except when calculating BCFs and there is insufficient biomass available for individual measurements.

(d) *Test conditions*—(1) *Test species* — (i) *Selection.* (A) The midge, *Chironomus tentans* or *C. riparius* shall be used in this test. Both species are widely distributed throughout the United States, and the larvae and adult flies can be cultured in the laboratory. The al portion of both species' life cycles is spent in a tunnel or case within the upper layers of benthic sediments of kes, rivers, and estuaries. Feeding habits of both species include both filter feeding and ingesting sediment particles.

(B) Second instar chironomids (≦ 10 days) of the same age and size are to be used in this test. Third and fourth instar are less desirable, as some evidence indicates they are less sensitive, at least to copper. Each instar is 4 to 7 days in duration.

(ii) *Acquisition.* (A) Chironomids to be used in this test should be cultured at the test facility. Adult flies are collected from the chironomid cultures and allowed to mate and lay egg masses. Two egg masses are collected and allowed to hatch. The larvae are fed daily. When the second instar stage (about 10 days after hatching) is reached, larvae are removed and placed in the test chambers. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test shall have originated from the same population (culture container) and be the same in age and size.

(B) Chironomids shall not be used in a test if:

*) During the final 48 hours of midge ling, obvious mortality is observed.

(2) The larvae are not in the second instar.

(iii) *Feeding.* (A) During the test, the chironomids should be fed the same diet and with the same frequency as that used for culturing and acclimation. All treatments and control(s) should receive, as near as reasonably possible, the same amount of food on a per-animal basis.

(B) The food concentration depends on the type used and the nutritional requirements of the midges. The latter in turn is dependent upon the stage of their development.

(iv) *Loading.* The number of test organisms placed in a test chamber should not affect the test results. Loading should not exceed 30 chironomids per liter per 24 hours in the flow-through test. Loading should not effect test concentrations or cause the DO concentration to fall below the recommended level.

(v) *Care and handling of test organisms.* (A) Chironomids should be cultured in dilution water under similar environmental conditions as those in the test. Food such as Tetra® Conditioning Food has been demonstrated to be adequate for chironomid cultures.

(B) Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and as quickly as possible. During culturing and acclimation, midges should be observed for any signs of stress, physical damage, and mortality. Dead and abnormal individuals shall be discarded. Organisms that are damaged or dropped during handling shall be discarded.

(C) Wide-bore, smooth glass tubes or pipets equipped with a rubber bulb can be used for transferring midges.

(vi) *Acclimation.* (A) Midges shall be maintained in 100 percent dilution water at the test temperature for at least 4 days prior to the start of the test. This is easily accomplished by culturing them in the dilution water at the test temperature. Chironomids shall be fed the same food during the test as is used for culturing and acclimation.

(B) During culturing and acclimation to the dilution water, midges should be maintained in facilities similar to those of the testing area.

(2) *Facilities*—(i) *General.* (A) Facilities needed to perform this test include:

(*1*) Containers for culturing and acclimating the chironomids;

(*2*) A mechanism for controlling and maintaining the water temperature during the culturing, acclimation, and test periods;

(*3*) Apparatus for straining particulate matter, removing gas bubbles, or aerating the water as necessary to ensure that the test solution flows regularly into and out of the container. Test chambers can be small aquaria capable of holding 3 liters of water or test solution, 5.7 liter clear glass battery jars, or 1 liter beakers made of borosilicate glass. Each chamber should be equipped with screened overflow holes, standpipes, or u-shaped notches covered with Nitex screen. Construction materials and commercially purchased equipment that may contact dilution water should not contain substances that can be leaked or dissolved into aqueous solutions in quantities that can alter the test results. Materials and equipment that contact test solutions should be chosen to minimize sorption of test substances; and

(*4*) Test chambers should be loosely covered to reduce the loss of test solution or dilution water by evaporation, and to minimize the entry of dust or other particulates into the solutions.

(ii) *Test substance delivery system.* (A) In the flow-through test, proportional diluters, metering pump systems or other suitable systems should be used to deliver the test substance to the test chambers.

(B) The test substance delivery system used shall be calibrated before and after each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system shall be checked twice daily during the test. The 24–hour flow rate through a test chamber shall be equal to at least five times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from any one test chamber to another or from one time to any other.

(iii) *Dilution water.* (A) Surface or ground water, reconstituted water, or dechlorinated tap water are acceptable as dilution water if chironomids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress. The quality of the dilution water should be constant and should meet the specifications in the following Table 2:

TABLE 2.—SPECIFICATIONS FOR DILUTION WATER

Substance	Maximum Concentration
Particulate matter	20 mg/L
Total organic carbon (TOC) or chemical oxygen demand (COD).	2 mg/L or 5 mg/L, respectively

TABLE 2.—SPECIFICATIONS FOR DILUTION WATER—Continued

Substance	Maximum Concentration
Boron, fluoride	100 µg/L
Un-ionized ammonia	10 µg/L
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc.	1 µg/L
Residual chlorine	3 µg/L
Cadmium, mercury, silver	100 ng/L
Total organophosphorus pesticides.	50 ng/L
Total organochlorine pesticides and polychlorinated biphenyls (PCBs) or Organic chlorine.	50 ng/L or 25 ng/L, respectively

(B) The water quality characteristics listed in Table 2 of paragraph (b)(2)(iii)(A) of this section shall be measured at least twice a year or when it is suspected that these characteristics may have changed significantly. If dechlorinated tap water is used, daily chlorine analysis shall be performed.

(C) If the diluent water is from a ground or surface water source, conductivity, hardness, alkalinity, pH, acidity, particulate matter, TOC or COD, and particulate matter shall be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass distilled or carbon filtered deionized water with conductivity of less than 1 microohm/cm is acceptable as the diluent for making reconstituted water.

(D) If the test substance is not soluble in water, an appropriate carrier such as triethylene glycol (CAS No. 112–27–6), dimethylformamide (CAS No. 68–12–2), or acetone (CAS No. 67–64–1) should be used. The concentration of such carriers should not exceed 0.1 mL/L.

(iv) *Cleaning of test system.* All test equipment and test chambers shall be cleaned before each test following standard laboratory procedures. Cleaning of test chambers may be necessary during the testing period.

(v) *Sediments.* (A) Sediments used in this test may contain low (< 1 percent) to high (>15 percent) amounts of organic carbon because they are derived from variable natural sediments. Prior to use, the sediments should be sieved to remove larger particles. They should be characterized for particle size distribution (sand, silt, clay percentages), percent water holding capacity, total organic and inorganic carbon, total volatile solids, COD, BOD, cation exchange capacity, redox potential (E_h), oils and greases, petroleum hydrocarbons, organophosphate pesticide concentrations, organochlorine pesticide [and polychlorinated biphenyl (PCB)] concentrations, toxic metal concentrations, and pH.

(B) The source of the sediments used in this test shall be known and the characteristics in paragraph (d)(2)(v)(A) of this section should be measured every time additional sediments are obtained. The sediments should not contain any endemic organisms, as these may be chironomid predators.

(C) Sediments should not be resuspended during the test.

(vi) *Sediment partition coefficient.* (A) The sediment or soil-water partition coefficient (K_p) is described as the ratio of the concentration of the test substance in the sediment (C_s) to the concentration in the water or interstitial water (C_w). This is expressed by the formula:

$$K_p = \frac{C_s}{C_w}$$

The resulting K_p values for the sediment or sediments tested are used to select test substance concentrations for the sediment test.

(B) The K_p value is equivalent or related to the sediment organic carbon sorption coefficient multiplied by the percent organic carbon content of the sediment.

(C) The sediment partition coefficient should be determined in triplicate for each sediment type at equilibrium by spiking with the radiolabeled test substance and shaking. Periodically, the test substance concentration in the water is measured radiometrically. The shaking and sampling is repeated until an equilibrium, as defined by the shape of the plotted desorption curve, is reached.

(vii) *Bioconcentration Factors.* BCFs shall be calculated for each part of the test. These values are computed as the amount of test substance present in the midge tissues divided by test substance concentrations in the water column, interstitial water, and sediments. At test termination, the chironomids remaining in each test concentration are analyzed for radiolabeled test substance.

(3) *Test parameters.* (i) Environmental conditions of the water contained in test chambers should be maintained as specified below:

(A) Temperature of 20 ± 1 °C for *C. tentans* and 22 ± 1 °C for *C. riparius*.

(B) DO concentration of the dilution water should be 90 percent of saturation or greater. The DO concentrations of the test solutions shall be 60 percent or greater of saturation, throughout the test. Aeration may be necessary, and if this is done, all treatment and control chambers should be given the same aeration treatment.

(C) A photoperiod of 16 hours light and 8 hours darkness with a 15 to 30 minute transition period.

(ii) Additional measurements include:

(A) The concentration of dissolved test substance (that which passes through a 0.45 micron filter) in the chambers should be measured during the test.

(B) At a minimum, the concentration of test substance should be measured as follows:

(1) In each chamber before the test.

(2) In each chamber on days 7 and 14 of the test.

(3) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

(C) Among replicate test chambers of a treatment concentration, the measured concentration of the test substance shall not vary by more than 20 percent at any time or 30 percent during the test.

(D) The dissolved oxygen concentration, temperature and pH shall be measured at the beginning of the test and on days 7 and 14 in each chamber.

(e) *Reporting.* The sponsor shall submit to the USEPA all data developed by the test that are suggestive and predictive of toxicity and all associated toxicologic manifestations. In addition to the reporting requirements prescribed in the GLPS (40 CFR part 792), the reporting of test data shall include the following:

(1) The name of the test, sponsor, testing laboratory, study director, principal investigator, and dates of testing.

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(2) A detailed description of the test substance including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and any carriers or other additives used and their concentrations.

(3) The source of the dilution water, its chemical characteristics (e.g., conductivity, hardness, pH, TOC or COD, and particulate matter) and a description of any pretreatment.

(4) The source of the sediment, its physical and chemical characteristics (e.g., particle size distribution, TOC, pesticide and metal concentrations), and a description of any pretreatment.

(5) Detailed information about the chironomids used as a stock, including the scientific name and method of verification, age, source, treatments, feeding history, acclimation procedures, and culture methods. The age (in days) and instar stage of the midges used in the test shall be reported.

(6) A description of the test chambers, the volume of solution in the chambers, and the way the test was begun (e.g., conditioning and test substance additions). The number of test organisms per test chamber, the number of replicates per treatment, the lighting, the test substance delivery system, flow rates expressed as volume additions per hours for the flow-through sub-chronic test, the method of feeding (manual or continuous), and type and amount of food.

(7) The concentration of the test substance in the water, interstitial water, and sediments in test chambers at times designated in the flow-through tests.

(8) The number and percentage of organisms that show any adverse effect in each test chamber at each observation period, and wet weights of midges in each test chamber at days 7 and 14.

(9) BCFs for all three parts of the test (i.e., overlying water or water column, sediment, and interstitial water modes of exposure).

(10) All chemical analyses of water quality and test substance concentrations, including methods, method validations and reagent blanks.

(11) The data records of the culture, acclimation, and test temperatures. Information relating to calculation of sediment (or soil-water) partition coefficients (K_p).

(12) Any deviation from this test guideline, and anything unusual about the test (e.g., diluter failure and temperature fluctuations).

(13) An LC50 value based on mortality an EC50 value based on adverse effects on growth (wet weights), with corresponding 95 percent confidence limits, when sufficient data are present for days 4, 7, and 14. These calculations should be made using the average measured concentration of the test substance.

(14) Concentration-response curves utilizing the average measured test substance concentration should be fitted to both number of midges that show adverse effects (mortality) and effects on growth or wet weights of midges at days 4, 7 and 14. A statistical test of goodness-of-fit should be performed and the results reported.

(15) The MATC to be reported is calculated as the geometric mean between the lowest measured test substance concentration that had significant ($P < 0.05$) effect and the highest measured test substance concentration that had no significant ($P > 0.05$) effect on days 4, 7, and 14 of the test. The criterion selected for MATC computation is the one which exhibits an effect (a statistically significant difference between treatment and control groups; $P < 0.05$) at the lowest test substance concentration for the shortest period of exposure. Appropriate statistical tests (analysis of variance and mean separation tests) should be used to test for significant test substance effects. The statistical tests employed and the results of these tests should be reported.

(f) *References.* For further background information on this test guideline the following references should be consulted:

(1) Adams, W. J., Kimerle, R. A., Mosher, R. G. "Aquatic safety assessment of chemicals sorbed to sediments." R. D. Cardwell, R. Purdy, and R. C. Bahner, eds. In: *Aquatic Toxicology and Hazard Assessment.* ASTM STP 854. American Society for Testing and Materials. (1985).

(2) Nebeker, A. V., Cairns, M. A., Wise, C. M. "Relative sensitivity of *Chironomus tentans* life stages to copper." *Environmental Toxicology and Chemistry* 3:151 158. (1984).

(3) Nebeker, A. V., Cairns, M. A., Gakstatter, J. H., Malueg, K. W., Schuytema, G. S., Krawczyk, D. F. "Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates." *Environmental Toxicology and Chemistry* 3:617–630. (1984).

c. By adding § 795.150 to read as follows:

§ 795.150 Earthworm toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 et seq.). The guideline sets forth the procedures and conditions for conducting this toxicity test. The U.S. Environmental Protection Agency (EPA) will use data from this test in assessing the hazard of a chemical to earthworms in the soil environment.

(b) *Definitions.* The definitions in section 3 of TSCA and the definitions in "Good Laboratory Practice Standards" (GLPS) (40 CFR part 792) apply to this guideline. The following definitions also apply:

Artificial soil means a defined dry weight mixture of 68 percent of No. 70 mesh silica sand, 20 percent kaolin clay, 10 percent sphagnum peat moss, and 2 percent calcium carbonate. These ingredients are weighed and mixed in the above proportions and moistened to 35 percent (by weight) with deionized/distilled water.

Behavioral symptoms are indicators of toxicity to earthworms such that a distinct difference in position in the test container can be identified, e.g., below surface or on the surface; writhing on the surface; stiffened and shortened on the surface or elongated and pulsing; or inactive below surface in a ball.

Clitellum means a glandular portion of the anterior epidermis, appearing as saddle-shaped or annular, usually differentiated externally by color.

Culture means the animals which are raised on-site or maintained under controlled conditions to produce test organisms through reproduction.

EC50 means that test substance concentration calculated from experimentally-derived growth or sublethal effects data that has affected 50 percent of a test population during continuous exposure over a specified period of time.

LC50 means that experimentally derived concentration of test substance that is estimated to kill 50 percent of a test population during continuous exposure over a specified period of time.

Lowest observed effect concentration (LOEC) means the lowest treatment (i.e., test concentration) of a test substance that is statistically different in adverse effect on a specific population of test organisms from that observed in controls.

Mature or adult worms means a condition of the worm exhibiting a clitellum in the anterior 1/3 of the body.

Mortality means the lack of movement by the test organism in response to a definite tactile stimulus to the anterior end. Also, because earthworms tend to disintegrate rapidly after death, the absence of organisms in the enclosed soil test container is considered to mean death has occurred

No observed effect concentration (NOEC) means the highest treatment (i.e., test concentration) of a test substance that shows no statistical difference in adverse effect on a specific population of test organisms from that observed in controls.

Pathological symptoms means toxic effects, such as surface lesions and mid-segmental swellings or general ulcerated areas on the surface of the earthworm.

Test mixture means the test substance/artificial soil mixtures which the earthworms are exposed to during the test.

Test substance means any compound used in artificial soils spiked for laboratory testing of toxicity.

(c) *Test procedures*—(1) *Summary of the test.* (i) Test chambers are filled with appropriate amounts of test mixtures.

(ii) This toxicity test may be done by placing earthworms in test chambers containing test mixtures and allowing earthworms to ingest this test mixture soil ad libitum.

(iii) Acclimated earthworms are introduced into the test and control chambers by stratified random assignment.

(iv) Earthworms in the test and control chambers shall be observed every 7 days and the findings shall be recorded and dead earthworms removed.

(v) The pH, temperature, and the concentration of the test mixtures shall be measured at 7 day intervals in each test chamber.

(vi) Initial weight of earthworm shall be between 300 to 600 grams per container.

(vii) Concentration-response curves, LC50, EC50, LOEC, NOEC values, and 95 percent confidence intervals for the test substance are developed from the data collected during the test.

(2) [Reserved]

(3) *Range-finding test.* (i) If the toxicity of the test substance is not already known, a range-finding test should be performed to determine the range of concentrations to be used in the definitive test.

(ii) The earthworms should be exposed (for at least 28 days) to a range of concentrations of the test substance (e. g., 0.1, 1.0, 10, 100, 1,000 mg/kg dry weight artificial soil).

(iii) Nominal concentrations are acceptable and no replication is required. If the LC50 value is > 1,000 mg test substance (100 percent active ingredient) per kiligram dry weight of artificial soil, the definitive test does not have to be done.

(4) *Definitive test.* (i) This test is designed to determine a concentration-mortality curve at 28 days and estimate the respective LC50, EC50, LOEC, NOEC values and 95 percent confidence intervals.

(ii) If data permit, the concentration-response curves, LC50, EC50, LOEC, NOEC values, and 95 percent confidence interval also should be determined for 7, 14, and 21 days.

(iii) This toxicity test uses earthworms which are maintained in direct contact with an artificial soil allowing earthworms to ingest contaminated soil ad libitum.

(iv) A minimum of 30 earthworms exposed to each of 5 or more test concentrations and e control shall be tested.

(v) Test concentrations should be chosen in a geometric series in which the ratio is between 1.5 and 2.0 mg/kg (e.g., 2, 4, 8, 16, 32, and 64 mg/kg). All test concentrations shall be based on milligram of test chemical (100 percent active ingredient) per kiligram of artificial soil (air-dry weight).

(vi) Ten earthworms per container of 200 g (dry weight) artificial soil shall be placed in three replicates for each concentration and control. The distribution of individual earthworms among the test chambers shall be randomized. Test concentrations in artificial soil shall be analyzed for test chemical concentrations prior to the start of the test and at days 7, 14, 21, and 28 as a minimum.

(vii) The living earthworms should be placed on the surface of the medium and the jar capped and secured without making an airtight seal.

(viii) Any changes in soil temperature should not exceed 3 °C per day or 1 °C per hour. Earthworms should be held for a minimum of 7 days at the test temperature prior to testing.

(ix) Every test shall include a negative control consisting of uncontaminated artificial soil, conditions, procedures, and earthworms from the same group used in the definitive test as shown, except that none of the test substance is added.

(x) The test duration is 28 days.

(5) *Test results.* (i) Death is the primary criterion used in this test guideline to evaluate the toxicity of the test substance.

(ii) In addition to death, weight loss, behavioral symptoms and pathological symptoms shall be recorded.

(iii) Each test and control chamber shall be checked for dead or affected earthworms and observations recorded 7, 14, 21, and 28 days after the beginning of the test or within 1 hour of the designated times. Missing earthworms shall be considered to have died.

(iv) Mortality is assessed by emptying the test medium on a glass or other inert surface, sorting earthworms from the test mixture and testing their reaction to a gentle mechanical stimulus. Any adverse effects (e.g., weight loss, behavioral or pathological symptoms) are noted and shall be reported. The medium is returned to each container.

(v) The 28–day test result shall be unacceptable if:

(A) More than 20 percent of control organisms die; or

(B) The total mean weight of the earthworms in the control containers declines significantly during the test (i.e. by 30 percent).

(vi) Mortality is checked and recorded at days 7, 14, 21, and 28.

(vii) The mortality data shall be used to calculate LC50 values and their 95 percent confidence limits, and to plot concentration-response curves at days 7, 14, 21, and 28.

(viii) The sublethal effects and growth (i.e., fresh weight) data shall be used to plot concentration-response curves, calculate EC50 values, and determine LOEC and NOEC values. Appropriate statistical methods (e.g., one-way analysis of variance and multiple comparison test) should be used to test for significant differences between treatment means and determine LOEC and NOEC.

(6) *Analytical measurements*— (i) *Artificial soil analysis.* During the test, the temperature and pH shall be measured in the artificial soil at the beginning of the test (0–hour), and every 7 days thereafter.

(ii) *Measurement of test substance.* (A) The concentration of test substance in artificial soil shall be measured at a minimum in each test chamber at the beginning (0–hour, before earthworms are added) and every 7 days thereafter.

(B) The analytical methods used to measure the amount of test substance in a sample should be validated before beginning the test. The accuracy of a method should be verified by a method such as using known additions. This involves adding a known amount of the test substance to three samples of artificial soil taken from the test chamber and the same number of earthworms as are used in the test. The measured concentration of the test substance in those samples should span the concentration range to be used in the test. Validation of the analytical method should be performed on at least two separate days prior to starting the test.

(C) An analytical method is not acceptable if likely degradation products of the test substance give positive or negative interferences, unless it is shown that such degradation

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products are not present in the test chambers during the test.

(D) In addition to analyzing samples of artificial soil, at least one reagent blank, containing all reagents used, should also be analyzed.

(E) The measured concentration of the test substance in artificial soil in any chamber during the test should not vary more than 50 percent from the measured concentration prior to initiation of the test; concentration measurements should be as described by Neuhauser et al., in paragraphs (f)(5) and (f)(6) of this section, or an equivalent method.

(F) The mean measured concentration of test substance in artificial soil (dry weight) should be used to plot all concentration-response curves and to calculate all LC50, EC50, LOEC, and NOEC values.

(G) The total carbon (TC) shall be determined as measured by the method of Plumb described in paragraph (f)(7) of this section, or an equivalent method.

(iii) *Numerical.* The statistical methods recommended for use in calculating the LC50 and EC50 values include probit, logit, moving average, and binomial.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* The test species for this test is the earthworm *Eisenia fetida andrei* (Bouche). The species identity of the test organism should be verified using appropriate taxonomic keys as described by Fender in paragraph (f)(2) of this section, or an equivalent method.

(ii) *Age and condition of earthworms.* (A) Adult earthworms, 300–600 mg, are to be used to start the test.

(B) Earthworms used in toxicity tests should be purchased from a commercial source that can verify the species. Once verified, cultures should be maintained at the test facility. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test should have originated from the same population (culture).

(C) All newly acquired earthworms should be quarantined and observed for at least 14 days prior to use in a test.

(D) Earthworms should not be used if they have been under stress from too much or a lack of moisture as described by Reinecke and Venter in paragraph (f)(8) of this section, or an equivalent method; excessive or inadequate food or temperature as described by Tomlin and Miller in paragraph (f)(11) of this section, or an equivalent method; pH variation as described by Satchell and Dottie in paragraph (f)(9) of this section, or an equivalent method; or crowding. Any of these conditions will produce earthworms that may not be healthy.

(iii) *Preparation.* Sufficient numbers of earthworms should be harvested and sorted to insure that healthy individuals are used for the test. Any animals that appear to be injured shall not be used in the test and must be discarded.

(iv) *Acclimation of test earthworms.* Adult earthworms should be handled with care. Earthworms should be held for a minimum of 7 days in uncontaminated soil at the test temperature prior to testing.

(v) *Feeding.* (A) Substrate food for culturing *Eisenia fetida andrei* should be saturated (water) alfalfa (*Medicago sativa*) pellets.

(B) The earthworms are not fed during the test period.

(2) *Facilities*—(i) *General.* Facilities needed to perform this test include:

(A) Apparatus for providing continuous lighting.

(B) Chambers for exposing test earthworms to the test substance.

(C) A mechanism for controlling and maintaining the artificial soil temperature and relative humidity during the holding, acclimation, and test periods.

(ii) *Construction materials.* (A) Construction materials and equipment that contact test mixtures shall not contain substances that can be leached or dissolved into artificial soil in quantities that can affect the test results. Material and equipment that contact test mixtures shall be chosen to minimize sorption of test substances. Hard glass jars are preferable and should be heated in an ashing oven between tests; soft glass jars shall be used only once.

(B) Polyethylene containers (rectangular dish pans measuring 32.5 X 27.5 X 12.5 cm) for culturing earthworms, a mechanism (e.g., environmental chamber) for maintaining temperature and relative humidity of the cultures during culturing, and separate facilities for testing are required.

(C) Testing containers (eg. 1 pint glass canning jars) and lids, and suitable balances to measure soil mixtures and sample weights shall also be used.

(D) Relative humidity should be maintained above 85 percent. An open pan of water can be used for this purpose to prevent moisture loss from the containers.

(iii) *Test chambers.* (A) One-pint (1-pt) glass canning jars or their equivalent should be used for testing.

(B) The lids should be reversed (i.e., turned upside down), loosely capped and secured without making an airtight seal to reduce evaporation and permit air exchange.

(iv) *Cleaning of test system.* The test chambers should be cleaned before each test following standard laboratory procedures. If soft glass is to be used it must only be used once and then thrown away.

(v) *Medium preparation.* (A) For each concentration tested and controls, enough artificial soil must be prepared by recipe to yield 270 g of artificial soil (wet weight) per replicate. A dry weight mixture of 68 percent of No. 70 mesh silica sand, 20 percent kaolin clay, and 10 percent sphagnum peat moss are mixed until evenly distributed.

(B) Up to 2 percent pulverized calcium carbonate may be added to adjust the soil pH to 6.5 ± 0.5.

(C) An appropriate amount of high purity water (e.g., 70 g per 200 g of dry soil) is added to the artificial soil and mixed with the artificial soil to raise the artificial soil moisture level to 35 percent by weight to yield a total weight of 810 g artificial soil at 35 percent moisture.

(D) Appropriate portions of the artificial soil are mixed thoroughly with appropriate amounts of test substance to yield three replicates for each test concentration. Each test mixture is divided into three equal quantities of about 270 g as determined by weight. Each portion is placed into a separate 1 pint jar and represents one replicate for exposing 10 earthworms at the same concentration. Three replicates for negative and, if necessary, solvent controls are prepared from untreated portions of the artificial soil mixture.

(E) If a solvent is used, the opened chambers are placed in a hood for 24 hours to evaporate the solvent prior to adding the earthworms.

(F) Prior to the addition of earthworms, a 10-g sample shall be removed from each replicate to measure pH and test concentrations.

(3) *Test parameters*— (i) *Loading.* The number of earthworms placed in a test chamber should not be so great as to affect the results of the test. The weight of the individual earthworms should be between 300 mg and 600 mg each. The earthworms are selected from the culture randomly into groups of 10. These groups are then randomly assigned to the test containers and then weighed such that they do not differ more than ± 10 percent among the replicates.

(ii) *Temperature.* (A) The test soil temperature shall be 22 ± 2 °C. as described by Edwards in paragraph (f)(1) of this section, or using an equivalent method.

(B) Temperature shall be measured and reported at the beginning of the test and on days 7, 14, 21, and 28. The temperature should be measured at least hourly in one test container.

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(iii) *Light.* (A) Replicates shall be illuminated continuously with incandescent or fluorescent lights as described by Edwards in paragraph (f)(1) of this section, or using an equivalent method.

(B) Light intensity shall be about 400 lux measured at the artificial soil surface.

(C) Light intensity shall be measured at least once during the test at the surface of the container and checked weekly in the test chambers.

(e) *Reporting.* (1) The sponsor shall submit all data developed by the test that are suggestive or predictive of toxicity and all concomitant gross toxicological manifestations. The reporting of test data shall include the following information:

(i) Test Background including the name of the sponsor, testing laboratory, principal investigator, and dates of testing.

(ii) A detailed description of the test chemical including its chemical identification (CAS No., trade name, common name,) source, lot number, composition (identity and concentration or major ingredients and major impurities), known physical and chemical properties, empirical formula, water solubility, vapor pressure, manufacturer, method of application, and any carriers or other additives used and their concentrations. The volume or mass of any carriers should be reported. An exact description of how the test substance has been mixed into the artificial soil.

(iii) Detailed information about the earthworms used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, and culture method.

(iv) A description of the test situation, especially if there was a deviation from this test guideline as described above in soil preparation (paragraph (d)(2)(v)(A) of this section), addition of the chemical, culturing of the test species, lighting, pH, temperature, replicates, or the number of organisms per container.

(v) A description of the test container used, its size, volume and weight of soil used in each container, number of test organisms per container, number of test containers per concentration, conditioning of the test container, description of the method of test chemical introduction into the test medium (e.g., as a powder), stock solution used or not, and time between mixing of the stock solution and introduction of the earthworms.

(vi) The concentrations in artificial soil at the beginning of the test and the actual concentrations of the test chemical (if measured) in the soil before (day 0), during (day 7, 14, 21) and upon the conclusion of the test (day 28) and the dates the analyses were performed.

(vii) The total organic carbon (TOC) of the soil mixture.

(2) The reported results shall include:

(i) The number and percentage of organisms that were killed or showed any adverse effects at each test concentration, including controls, in each test jar at each observation period.

(ii) Concentration response curves fitted to mortality data at 7, 14, 21, and 28-day periods. A statistical test of goodness-of-fit shall be performed and reported.

(iii) The LC50/EC50 values and the 95 percent confidence limits using the mean measured test concentration and the methods used to calculate both the LC50/EC50: also the LOEC and NOEC values and the confidence intervals by the Trimmed Spearman-Karber method as described by Hamilton et al., in paragraph (f)(3) of this section, or an equivalent method. The probit technique should follow the methods described by Weber et al., in paragraph (f) (12) of this section, or an equivalent method. Appropriate statistical methods (e.g., one-way analysis of variance and multiple comparison test) should be used to test for significant differences between treatment and determine the LOEC and NOEC.

(iv) All chemical analyses of test material including methods, method validations, and reagent blanks.

(v) The data records for the culture and lighting.

(vi) Moisture content for the test mixture at start of test.

(vii) The pH and temperature values at start of test and on days 7, 14, 21, and 28 of the test.

(viii) Any deviation from this test guideline and anything unusual about the test (e.g., equipment failure, fluctuations in temperature, pH, or other environmental conditions).

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Edwards, C. A. "Report of the second stage in development of a standardized laboratory method for assessing the toxicity of chemical substances to earthworms," The Artificial Soil Test. DG X1/AL/82/43, Revision 4 (1984).

(2) Fender, W. M. "Earthworms of the Western United States," Part 1. Lumbricidae, *Megadrilogica*, 4: 93–129 (1985).

(3) Hamilton, M. A., Russo, R. C., and Thurston, R. V. "Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays," *Environmental Science and Toxicology* 11 (7): 714–717 (1977). Correction: Ibid 12: 417 (1978).

(4) Hartenstein, R., Neuhauser, E. F., and Kaplan, D. L. "Reproductive potential of the earthworm *Eisenia foetida*.", 43: *Oecologia*, 329–340 (1979).

(5) Neuhauser, E. F., Loehr, R. C., and Malecki, M. R. "Contact and artificial soil tests using earthworms to evaluate the impact of wastes in soils." In: Hazardous and Industrial Solid Waste Testing: Fourth Symposium, ASTM STP 886. J.K. Petros, Jr. and R. A. Conway, eds., (American Society for Testing and Materials: Philadelphia, PA. 1986) pp. 192–203.

(6) Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. "The toxicity of selected organic chemicals to the earthworm *Eisenia fetida*." *Journal of Environmental Quality*, 14: 383–388 (1985).

(7) Plumb, R.H., Jr. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE–81–1, prepared by Great Lakes Labroratory, State University College at Buffalo, Buffalo, NY., for the U.S. Environmental Protection Agency/ Corp of Engineers Technical Committee on Criteria for Dredged and Fill Material. U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, MS. (1981)

(8) Reinecke, A.J. and Venter, J. M. "Moisture preferences, growth and reproduction of the compost worm *Eisenia fetida* (Oligochaeta)," *Biology of Fertilty Soils*, 3: 135–141 (1987).

(9) Satchell, J.E. and Dottie, D. J. "Factors affecting the longevity of earthworms stored in peat," *Journal of Applied Ecology*, 21: 285–291 (1984).

(10) Stafford, E. A. and Edwards, C. A. "Comparison of heavy metal uptake by *Eisenia foetida* with that of other common earthworms", Final Technical Report. Entomology Department, Rothamsted Experiment Station, Harpenten, Herts. ALS 2JQ, U.K. U.S. Army Contract DAJA 45–84–0027 (1985).

(11) Tomlin, A. D. and Miller, J. J. "Development and fecundity of the manure worm, *Eisenia foetida* (Annelida:Lumbricidae), under laboratory conditions." In: D.L.Dindal (ed.), "Soil Biology as Related to Land Use Practices." Proc. 7th Internat. Soil Zool. Coll. of ISSS. EPA, Washington, DC., pp 673–678 (1980).

(12) Weber, C. I., Horning, W. B., II, Klemm, D. J., Neiheisel, T. W., Lewis, P. A., Robinson, E. L., Menkedick, J. R., Kessler, F. A. "Short-term methods for estimating the chronic toxicity of effluents and surface waters to marine

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and freshwater organisms," 2nd Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental P[illegible]ction Agency, Cincinnati, OH (600/4[illegible],028) (1988).

2. In part 798

PART 798 — [AMENDED]

a. By revising the authority citation for part 798 to read as follows:

Authority: 15 U.S.C. 2601, 2603

b. By revising § 798.3320 to read as follows:

§ 798.3320 Combined chronic toxicity/oncogenicity.

(a) *Purpose.* The objective of a combined chronic toxicity/oncogenicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. The application of this guideline shall generate data which identify the majority of chronic and oncogenic effects and determine dose-response relationships. The design and conduct shall allow for the detection of neoplastic effects and a determination of oncogenic potential as well as general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

Test procedures)—(1) *Animal selection*—(i) *Species and strain.* Preliminary studies providing data on acute, subchronic, and metabolic responses shall have been carried out to permit an appropriate choice of animals (species and strain). As discussed in other guidelines, the mouse and rat have been most widely used for assessment of oncogenic potential, while the rat and dog have been most often studied for chronic toxicity. The rat is the species of choice for combined chronic toxicity and oncogenicity studies. The provisions of this guideline are designed primarily for use with the rat as the test species. If other species are used, the tester shall provide justification/reasoning for their selection. The strain selected shall be susceptible to the oncogenic or toxic effect of the class of substances being tested, if known, and provided it does not have a spontaneous background too high for meaningful assessment. Commonly used laboratory strains shall be employed.

(ii) *Age.* (A) Dosing of rats shall begin as soon as possible after weaning, ideally before the rats are 6 weeks old, but in no case more than 8 weeks old.

(B) At commencement of the study, the weight variation of animals used sh[illegible] not exceed ± 20 percent of the m[illegible] weight for each sex.

(C) Studies using prenatal or neonatal animals may be recommended under special conditions.

(iii) *Sex.* (A) Equal numbers of animals of each sex shall be used at each dose level.

(B) The females shall be nulliparous and nonpregnant.

(iv) *Numbers.* (A) At least 100 rodents (50 females and 50 males) shall be used at each dose level and concurrent control for those groups not intended for early sacrifice. At least 40 rodents (20 females and 20 males) shall be used for satellite dose group(s) and the satellite control group. The purpose of the satellite group is to allow for the evaluation of pathology other than neoplasia.

(B) If interim sacrifices are planned, the number of animals shall be increased by the number of animals scheduled to be sacrificed during the course of the study.

(C) The number of animals at the termination of each phase of the study should be adequate for a meaningful and valid statistical evaluation of long term exposure. For a valid interpretation of negative results, it is essential that survival in all groups not fall below 50 percent at the time of termination.

(2) *Control groups.* (i) A concurrent control group (50 females and 50 males) and a satellite control group (20 females and 20 males) are recommended. These groups shall be untreated or sham treated control groups or, if a vehicle is used in administering the test substance, vehicle control groups. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are recommended. Animals in the satellite control group shall be sacrificed at the same time the satellite test group is terminated.

(ii) In special circumstances such as inhalation studies involving aerosols or the use of an emulsifier of uncharacterized biological activity in oral studies, a concurrent negative control group shall be utilized. The negative control group shall be treated in the same manner as all other test animals, except that this control group shall not be exposed to the test substance or any vehicle.

(iii) The use of historical control data (i.e., the incidence of tumors and other suspect lesions normally occurring under the same laboratory conditions and in the same strain of animals employed in the test) is desirable for assessing the significance of changes observed in exposed animals.

(3) *Dose levels and dose selection.* (i) For risk assessment purposes, at least three dose levels shall be used, in addition to the concurrent control group. Dose levels should be spaced to produce a gradation of effects.

(ii) The highest dose level in rodents should elicit signs of toxicity without substantially altering the normal life span by effects other than tumors.

(iii) The lowest dose level should produce no evidence of toxicity. However, where there is a usable estimation of human exposure, the lowest dose level should exceed this even though this dose level may result in some signs of toxicity.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used the dose levels should be spaced to produce a gradation of toxic effects.

(v) For rodents, the incidence of fatalities in low and intermediate dose groups and in the controls should be low to permit a meaningful evaluation of the results.

(vi) For chronic toxicological assessment, a high dose treated satellite and a concurrent control satellite group shall be included in the study design. The highest dose for satellite animals should be chosen so as to produce frank toxicity, but not excessive lethality, in order to elucidate a chronic toxicological profile of the test substance. If more than one dose level is selected for satellite dose groups, the doses should be spaced to produce a gradation of toxic effects.

(4) *Exposure conditions.* The animals are dosed with the test substance ideally on a 7 day per week basis over a period of at least 24 months for rats, and 18 months for mice and hamsters, except for the animals in the satellite groups which shall be dosed for 12 months.

(5) *Observation period.* It is necessary that the duration of the oncogenicity test comprise the majority of the normal life span of the animals to be used. It has been suggested that the duration of the study should be for the entire lifetime of all animals. However, a few animals may greatly exceed the average lifetime and the duration of the study may be unnecessarily extended and complicate the conduct and evaluation of the study. Rather, a finite period covering the majority of the expected life span of the strain is preferred since the probability is high that, for the great majority of chemicals, any induced tumors will occur within such an observation period. The following guidelines are recommended:

(i) Generally, the termination of the study shall be at 18 months for mice and hamsters and 24 months for rats; however, for certain strains of animals

with greater longevity and/or low spontaneous tumor rate, termination shall be at 24 months for mice and hamsters and at 30 months for rats. For longer time periods, and where any other species are used, consultation with the Agency in regard to duration of the test is advised.

(ii) However, termination of the study is acceptable when the number of survivors of the lower doses or of the control group reaches 25 percent. In the case where only the high dose group dies prematurely for obvious reasons of toxicity, this shall not trigger termination of the study.

(iii) The satellite groups and the concurrent satellite control group shall be retained in the study for at least 12 months. These groups shall be scheduled for sacrifice for an estimation of test-substance-related pathology uncomplicated by geriatric changes.

(6) *Administration of the test substance.* The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) *Oral studies.* (A) The animals shall receive the test substance in their diet, dissolved in drinking water, or given by gavage or capsule for a period of at least 24 months for rats and 18 months for mice and hamsters.

(B) If the test substance is administered in the drinking water, or mixed in the diet, exposure shall be continuous.

(C) For a diet mixture, the highest concentration should not exceed 5 percent.

(ii) *Dermal studies.* (A) The animals are treated by topical application with the test substance, ideally for at least 6 hours per day.

(B) Fur should be clipped from the dorsal area of the trunk of the test animals. Care should be taken to avoid abrading the skin which could alter its permeability.

(C) The test substance shall be applied uniformly over a shaved area which is approximately 10 percent of the total body surface area. With highly toxic substances, the surface area covered may be less, but as much of the area as possible shall be covered with as thin and uniform a film as possible.

(D) During the exposure period, the test substance may be held, if necessary, in contact with the skin with a porous gauze dressing and nonirritating tape. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

(iii) *Inhalation studies.* (A) The animals shall be tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour and to ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule, to ensure stability of a chamber atmosphere, the total "volume" of the test animals shall not exceed 5 percent of the volume of the test chamber. Alternatively, oronasal, head only, or whole body individual chamber exposure may be used.

(B) The temperature at which the test is performed should be maintained at 22 °C (± 2°). Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(C) Food and water shall be withheld during each daily 6-hour exposure period.

(D) A dynamic inhalation system with a suitable analytical concentration control system shall be used. The rate of air flow shall be adjusted to ensure that conditions throughout the equipment are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(7) *Observation of animals.* (i) Each animal shall be handled and its physical condition appraised at least once each day.

(ii) Additional observations shall be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Clinical signs and mortality should be recorded for all animals. Special attention shall be paid to tumor development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumor shall be recorded.

(iv) Body weights shall be recorded individually for all animals once a week during the first 13 weeks of the test period and at least once every 4 weeks thereafter, unless signs of clinical toxicity suggest more frequent weighings to facilitate monitoring of health status.

(v) When the test substance is administered in the food or drinking water, measurements of food or water consumption, respectively, shall be determined weekly during the first 13 weeks of the study and then at approximately monthly intervals unless health status or body weight changes dictate otherwise.

(vi) At the end of the study period, all survivors are sacrificed. Moribund animals shall be removed and sacrificed when noticed.

(8) *Physical measurements.* For inhalation studies, measurements or monitoring should be made of the following:

(i) The rate of airflow shall be monitored continuously, but shall be recorded at intervals of at least once every 30 minutes.

(ii) During each exposure period the actual concentrations of the test substance shall be held as constant as practicable, monitored continuously and recorded at least three times during the test period: At the beginning, at an intermediate time and at the end of the period.

(iii) During the development of the generating system, particle size analysis shall be performed to establish the stability of aerosol concentrations. During exposure, analyses shall be conducted as often as necessary to determine the consistency of particle size distribution and homogeneity of the exposure stream.

(iv) Temperature and humidity shall be monitored continuously, but should be recorded at intervals of at least once every 30 minutes.

(9) *Clinical examinations.* (i) The following examinations shall be made on at least 20 rodents of each sex per dose level:

(A) Certain hematology determinations (e.g., hemoglobin content, packed cell volume, total red blood cells, total white blood cells; platelets, or other measures of clotting potential) shall be performed at termination and shall be performed at 3 months, 6 months and at approximately 6 month intervals thereafter (for those groups on test for longer than 12 months) on blood samples collected from 20 rodents per sex of all groups. These collections shall be from the same animals at each interval. If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals shall be performed. A differential blood count shall be performed on samples from animals in the highest dosage group and the controls. Differential blood counts shall be performed for the next lower group(s) if there is a major discrepancy between the highest group and the controls. If hematological effects were noted in the

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subchronic test, hematological testing shall be performed at 3, 6, 12, 18 and 24 months for a two-year study.

(B) Certain clinical biochemistry determinations on blood shall be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), near the middle and at the end of the test period. Blood samples shall be drawn for clinical measurements from at least 10 rodents per sex of all groups; if possible, these shall be from the same rodents at each time interval. Test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance and signs of clinical toxicity. Suggested chemical determinations: Calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, blood urea nitrogen, albumen, creatinine phosphokinase, total cholesterol, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects.

(ii) The following shall be performed on at least 10 rodents of each sex per dose level:

(A) Urine samples from the same rodents at the same intervals as the hematological examination in paragraph (c)(9)(i)(A) of this section, shall be collected for analysis. The following determinations shall be made from either individual animals or on a pooled sample/sex/group for rodents: appearance (volume and specific gravity), protein, glucose, ketones, bilirubin, occult blood (semi-quantitatively) and microscopy of sediment (semi-quantitatively).

(B) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, shall be made prior to the administration of the test substance and at the termination of the study. If changes in the eyes are detected, all animals shall be examined.

(10) *Gross necropsy.* (i) A complete gross examination shall be performed on all animals, including those which died during the experiment or were killed in moribund conditions.

(ii) The liver, kidneys, adrenals, brain and gonads shall be weighed wet, as soon as possible after dissection to avoid drying. For these organs, at least 10 rodents per sex per group shall be weighed.

(iii) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: All gross lesions and tumors; brain—including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; lungs; trachea; heart; sternum and/or femur with bone marrow; salivary glands; liver; spleen; kidneys, adrenal; esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph nodes; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and if present, seminal vesicles); female mammary gland; aorta; gall bladder (if present); skin; musculature; peripheral nerve; spinal cord at three levels—cervical, midthoracic, and lumbar; and eyes. In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx and paranasal sinuses shall be examined and preserved. In dermal studies, skin from sites of skin painting shall be examined and preserved.

(iv) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is considered essential for appropriate and valid histopathological examination.

(v) If other clinical examinations are carried out, the information obtained from these procedures shall be available before microscopic examination, since they may provide significant guidance to the pathologist.

(11) *Histopathology.* (i) The following histopathology shall be performed:

(A) Full histopathology on the organs and tissues, listed in paragraph (b)(10)(i) through (b)(10)(iii) of this section, of all non-rodents, of all rodents in the control and high dose groups and of all rodents that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target organs in all animals.

(D) Lungs, liver and kidneys of all animals. Special attention to examination of the lungs of rodents shall be made for evidence of infection since this provides an assessment of the state of health of the animals.

(ii) If excessive early deaths or other problems occur in the high dose group compromising the significance of the data, the next dose level shall be examined for complete histopathology.

(iii) In case the results of the experiment give evidence of substantial alteration of the animals' normal longevity or the induction of effects that might affect a toxic response, the next lower dose level shall be examined for complete histopathology.

(iv) An attempt shall be made to correlate gross observations with microscopic findings.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Data shall be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, shall be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of study results.* (i) The findings of a combined chronic toxicity/oncogenicity study shall be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects, the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(iii) For a negative test to be acceptable, it shall meet the following criteria: No more than 10 percent of any group is lost due to autolysis, cannibalism, or management problems; and survival in each group is no less than 50 percent at 18 months for mice and hamsters and at 24 months for rats.

(3) *Test report.* (i) In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information shall be reported:

(A) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(*1*) Number of animals dying.

(*2*) Number of animals showing signs of toxicity.

(*3*) Number of animals exposed.

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(B) *Individual animal data.* (*1*) Time of death during the study or whether animals survived to termination.

(*2*) Time of observation of each abnormal sign and its subsequent course.

(*3*) Body weight data.

(*4*) Food and water consumption data, when collected.

(*5*) Results of ophthalmological examination, when performed.

(*6*) Hematological tests employed and all results.

(*7*) Clinical biochemistry tests employed and all results.

(*8*) Necropsy findings.

(*9*) Detailed description of all histopathological findings.

(*10*) Statistical treatment of results where appropriate.

(*11*) Historical control data, if taken into account.

(ii) In addition, for inhalation studies the following shall be reported:

(A) *Test conditions.* (*1*) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(*2*) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size shall be described.

(B) *Exposure data.* These shall be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and shall include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*4*) Actual concentration in test breathing zone.

(*5*) Particle size distribution (e.g., median aerodynamic diameter of particles with standard deviation from the mean).

(d) *References.* For additional background information on this test guideline the following references should be consulted.

(1) D'Aguanno, W. "Drug Safety Evaluation—Pre-Clinical Considerations." *Industrial Pharmacology: Neuroleptics.* Vol. I. S. Fielding and H. Lal, eds. Mt. Kisco, New York: Futura Publishing Co., pp. 317–332 (1974).

(2) Department of Health and Welfare. "The Testing of Chemicals for Carcinogenicity, Mutagenicity, Teratogenicity." Minister of Health and Welfare. Canada: Department of Health and Welfare (1975).

(3) Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation: Panel on Carcinogenesis. "Report on Cancer Testing in the Safety of Food Additives and Pesticides." *Toxicology and Applied Pharmacology.* 20:419–438 (1971).

(4) International Union Against Cancer. "Carcinogenicity Testing." IUCC Technical Report Series Vol. 2, Ed. I. Berenblum. Geneva: International Union Against Cancer (1969).

(5) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances", A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(6) National Cancer Institute. "Report of the Subtask Group on Carcinogen Testing to the Interagency Collaborative Group on Environmental Carcinogenesis." Bethesda, MD: United States National Cancer Institute (1976).

(7) National Center for Toxicological Research. "Report of Chronic Studies Task Force Research Committee. Appendix B", Rockville, MD: National Center for Toxicological Research (1972).

(8) Page, N.P. "Chronic Toxicity and Carcinogenicity Guidelines," *Journal Environmental Pathology and Toxicology.* 1:161–182 (1977).

(9) Page, N.P. "Concepts of a Bioassay Program in Environmental Carcinogenesis". *Advances in Modern Toxicology* Vol. 3, ed. Kraybill and Mehlman. Washington, D.C.: Hemisphere Publishing Corp., p. 67–171 (1977).

(10) World Health Organization. "Principles for the Testing and Evaluation of Drugs for Carcinogenicity", WHO Technical Report Series No. 426. Geneva: World Health Organization (1969).

(11) World Health Organization. "Guidelines for Evaluation of Drugs for Use in Man", WHO Technical Report Series No. 563. Geneva: World Health Organization (1975).

(12) World Health Organization. "Part I. Environmental Health Criteria 6", *Principles and Methods for Evaluating the Toxicity of Chemicals.* Geneva: World Health Organization (1978).

(13) World Health Organization. "Principles for Pre-Clinical Testing of Drug Safety", WHO Technical Report Series No. 341. Geneva: World Health Organization (1966).

3. In part 799:

PART 799 — [AMENDED]

a. By revising the authority citation for part 799 to read as follows:

Authority: 15 U.S.C. 2601, 2603, 2611, 2625.

b. By adding § 799.5110 to read as follows:

§ 799.5110 Brominated flame retardants.

(a) *Identification of test substances.* (1) Pentabromodiphenyl ether (PBDPE; CAS No. 32534–81–9), octabromodiphenyl ether (OBDPE; CAS No. 32536–52–0), decabromodiphenyl ether (DBDPE; CAS No. 1163–19–5), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE; CAS No. 37853–59–1), and hexabromocyclododecane (HBCD; CAS No. 3194–55–6) shall be tested in accordance with this section.

(2) PBDPE, OBDPE, DBDPE, BTBPE, and HBCD of at least 98 percent purity shall be used as the test substance. For the three diphenyl ethers, "purity" refers to freedom from substances that do not fit the description "brominated diphenyl ethers."

(3) PBDPE as the test substance shall contain at least 56 percent pentabromodiphenyl ether isomers, not more than 25 percent tetrabromodiphenyl ether isomers, and not more than 25 percent hexabromodiphenyl ether isomers. In addition, PBDPE shall not contain more than 10 percent tri- (or lower) brominated diphenyl ether isomers, and also not more than 10 percent hexa(or higher) brominated diphenyl ether isomers.

(4) OBDPE as the test substance shall contain at least 30 percent octabromodiphenyl ether isomers, not more than 45 percent heptabromodiphenyl ether isomers, and not more than 15 percent nonabromodiphenyl ether isomers. In addition, OBDPE shall not contain more than 15 percent hexa- (or lower) brominated diphenyl ether isomers, and also not more than 5 percent deca- (or higher) brominated diphenyl ether isomers.

(5) DBDPE as the test substance shall contain at least 98 percent decabromodiphenyl ether.

(6) Congenerically pure PBDPE shall contain at least 98 percent pentabromodiphenyl ether isomers.

(7) Congenerically pure OBDPE shall contain at least 98 percent octabromodiphenyl ether isomers.

(b) *Persons required to submit study plans, conduct tests and submit data.* All persons who manufacture (including import) or process or intend to manufacture or process PBDPE, OBDPE, DBDPE, BTBPE, or HBCD, other than as an impurity, after (insert date 44 days after date of publication of the final test rule in the Federal Register) to the end of the reimbursement period shall submit letters of intent to conduct testing, submit study plans, conduct tests, and submit data, or submit exemption applications as specified in this section, subpart A of this part and parts 790 and 792 of this chapter for single-phase rulemaking, for the substances they manufacture.

(c) *Health effects testing*—(1) *Mutagenic effects—gene mutation*—(i)

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Required testing. (A) Gene mutation assays in the *Salmonella typhimurium* ... stidine reversion system shall be ...nducted with OBDPE in accordance with § 798.5265 of this chapter.

(B) Gene mutation assays in somatic cells in culture shall be conducted with PBDPE, OBDPE, BTBPE, and HBCD in accordance with § 798.5300 of this chapter.

(C) A sex-linked recessive lethal test in *Drosophila melanogaster* shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE or HBCD in accordance with § 798.5275 of this chapter for any of these substances that produces a positive result in either the *Salmonella* assay conducted on OBDPE, DBDPE, and HBCD pursuant to paragraph (c)(2)(i)(A) of this section or the somatic cells in culture assay conducted on PBDPE, OBDPE, DBDPE, BTBPE, and HBCD pursuant to paragraph (c)(2)(i)(B) of this section.

(D) A mouse visible specific locus test (MVSL) or a mouse biochemical specific locus (MBSL) test shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, or HBCD in accordance with § 798.5200 or § 798.5195, respectively, for whichever of these substances produces a positive result in the sex-linked recessive lethal test in *Drosophila melanogaster* conducted pursuant to paragraph ...(2)(i)(C) of this section.

(ii) *Reporting requirements.* (A) Mutagenic effects - gene mutation tests shall be conducted and the final reports submitted to EPA as follows:

(*1*) Gene mutation in *Salmonella*, 9 months after the effective date.

(*2*) Gene mutation in somatic cells in culture, 10 months after the effective date.

(*3*) *Drosophila* sex-linked recessive lethal, 22 months after the effective date.

(*4*) Mouse specific locus, within 51 months of the date of EPA's notification of the test sponsor by certified letter that testing shall be initiated.

(B) Progress reports shall be submitted to EPA every 6 months beginning 6 months after the effective date for the gene mutation tests in *Salmonella* and gene mutation tests in somatic cells in culture; for the *Drosophila* test, beginning 6 months after the date the final report is submitted for the gene mutation in somatic cells in culture test; and for the mouse specific locus test, beginning 6 months after the date of EPA's notification of the test sponsor that testing shall be initiated.

(2) *Mutagenic effects—chromosomal aberrations* — (i) *Required testing.* (A) *In vivo* cytogenetic assays shall be ...onducted with PBDPE, OBDPE, DBDPE, ...TBPE, and HBCD in accordance with § § 798.5385 or 798.5395 of this chapter.

(B) A dominant lethal assay shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, or HBCD in accordance with § 798.5450 of this chapter, for any of these substances that produces a positive result in the *in vivo* cytogenetic assay conducted pursuant to paragraph (c)(2)(i)(A) of this section.

(C) A heritable translocation assay shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, or HBCD in accordance with § 798.5460 of this chapter, for any of these substances that produces a positive result in the dominant lethal assay conducted pursuant to paragraph (c)(3)(i)(B) of this section.

(ii) *Reporting requirements.* (A) Mutagenic effects-chromosomal aberration testing shall be completed and the final reports submitted to EPA as follows:

(*1*) *In vivo* cytogenetics, within 14 months after the effective date; and dominant lethal assay, within 36 months after the effective date.

(*2*) Heritable translocation assay, within 25 months of the date of EPA's notification of the test sponsor by certified letter that testing shall be initiated.

(B) Progress reports shall be submitted to EPA every 6 months beginning as follows:

(*1*) For the *in vivo* cytogenetics assay, 6 months after the effective date.

(*2*) For the dominant lethal assay, beginning 6 months after the date the final report is submitted for the *in vitro* cytogenetics test.

(*3*) For the heritable translocation assay, beginning 6 months after the date of EPA's notification of the test sponsor that testing shall be initiated.

(3) *Subchronic toxicity* — (i) *Required testing.* Subchronic toxicity testing shall be conducted by gavage with HBCD in accordance with § 798.2650 of this chapter.

(ii) *Reporting requirements.* (A) The required subchronic toxicity test shall be completed and the final reports submitted to EPA within 18 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 6 months after the effective date until the final report is submitted.

(4) *Neurotoxicity*—(i) *Required testing*—(A) *Functional observational battery. (1)* A functional observational battery test shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with § 798.6050 of this chapter except for the provisions in paragraphs (d)(4)(ii), (d)(5), and (d)(6) of § 798.6050.

(*2*) For the purpose of this section the following provisions also apply:

(*i*) *Lower doses.* The data from the lower doses shall show either graded dose-dependent effects or no neurotoxic (behavioral) effects at any dose tested.

(*ii*) *Duration and frequency of exposure.* For the acute testing, animals shall be treated once. For the subchronic testing, animals shall be treated 5 consecutive days per week for a 90–day period.

(*iii*) *Route of exposure.* Animals shall be exposed to PBDPE, OBDPE, DBDPE, BTBPE, and HBCD by gavage administration.

(B) *Motor activity.* (*1*) Motor activity testing shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with § 798.6200 of this chapter except for the provisions in paragraphs (d)(4)(ii), (d)(5), and (d)(6) of § 798.6200.

(*2*) For the purpose of this section, the following provisions also apply:

(*i*) *Lower doses.* The data from the lower doses shall show either graded dose-dependent effects or no neurotoxic (behavioral) effects at any dose tested.

(*ii*) *Duration and freguency of exposure.* For the acute testing, animals shall be treated once. For the subchronic testing animals shall be treated 5 consecutive days per week for a 90–day period.

(*iii*) *Route of exposure.* Animals shall be exposed to PBDPE, OBDPE, DBDPE, BTBPE, and HBCD by gavage administration.

(C) *Neuropathology.* (*1*) Neuropathology testing shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD administered by gavage in accordance with § 798.6400 of this chapter except for the provisions in paragraphs (d)(4)(ii), (d)(5), (d)(6) and (d)(8)(iv)(C) of § 798.6400.

(*2*) For the purpose of paragraph (c)(6)(i)(C) of this section, the following provisions also apply:

(*i*) *Lower doses.* The data from the lower doses shall show either graded dose-dependent effects or no neurotoxic (behavioral) effects at any dose tested.

(*ii*) *Duration and freguency of exposure.* For the acute testing, animals shall be treated once. For the subchronic testing animals shall be treated 5 consecutive days per week for a 90–day period.

(*iii*) *Route of exposure.* Animals shall be exposed to PBDPE, OBDPE, DBDPE, BTBPE, and HBCD by gavage administration.

(*iv*) *Clearing and embedding.* After dehydration, tissue specimens shall be cleared with xylene and embedded in wax or plastic medium except for the sural nerve which should be embedded in plastic. Multiple tissue specimens (e.g.

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brain, cord, ganglia) may be embedded together in one single block for sectioning. All tissue blocks shall be labelled to provide unequivocal identification. Plastic embedding should follow the method described by Spencer, et al., in paragraph (f) of this section, or an equivalent method.

(ii) *Reporting requirements.* (A) The functional observational battery, motor activity, and neuropathology testing with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD shall be completed and the final reports submitted to EPA within 21 months of the effective date.

(B) Progress reports shall be submitted every 6 months beginning 6 months after the effective date until the final report is submitted.

(5) *Reproductive toxicity*—(i) *Required testing.* A reproductive toxicity test shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD by gavage in accordance with § 798.4700 of this chapter.

(ii) *Reporting requirements.* (A) The reproductive toxicity test for PBDPE, DBDPE, BTBPE, and HBCD shall be completed and the final reports submitted to EPA within 29 months of the effective date. The reproductive toxicity test for OBDPE shall be completed and the final report submitted to EPA within 29 months of the test sponsor's receipt of a certified letter from EPA specifying that a reproductive toxicity test for OBDPE be initiated.

(B) Progress reports shall be submitted to EPA every 6 months beginning 6 months after the effective date for PBDPE, DBDPE, BTBPE, HBCD, and, for OBDPE, beginning 6 months after the test sponsor's receipt of a certified letter specifying that a reproductive toxicity test be initiated until the final report is submitted.

(6) *Developmental toxicity*—(i) *Required testing.* (A) Developmental toxicity testing in two species, a rat and nonrodent, shall be conducted with PBDPE, OBDPE, and HBCD by gavage in accordance with § 798.4900 of this chapter.

(B) Developmental toxicity testing in one non-rodent species shall be conducted with DBDPE and BTBPE by gavage in accordance with § 798.4900 of this chapter.

(ii) *Reporting requirements.* (A) The developmental toxicity testing shall be completed and the final reports submitted to EPA within 12 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 6 months after the effective date until the final report is submitted.

(7) *Oncogenicity*—(i) *Required testing.* (A) Oncogenicity testing shall be conducted in mice with PBDPE, OBDPE, and BTBPE by gavage in accordance with § 798.3300 of this chapter.

(B) Oncogenicity testing shall also be conducted in both rats and mice with HBCD by gavage in accordance with § 798.3300 of this chapter if a positive result is obtained in any one of the following mutagenicity tests and EPA notifies the sponsor by certified letter that testing shall be initiated:

(*1*) The gene mutation somatic cells in culture assay conducted pursuant to paragraph (c)(1)(i)(B) of this section.

(*2*) The sex-linked recessive lethal assay in *Drosophila melanogaster* conducted pursuant to paragraph (c)(1)(i)(C) of this section.

(*3*) The *in vivo* cytogenetics assay conducted pursuant to paragraph (c)(2)(i)(A) of this section.

(C) Criteria for positive test results are established in 40 CFR 798.5395, 798.5385, 798.5300, and 798.5275 of this chapter, respectively.

(ii) *Reporting requirements.* (A) The oncogenicity testing for PBDPE, OBDPE, and BTBPE shall be completed and the final reports submitted to EPA within 53 months of the effective date. The oncogenicity testing for HBCD, if required, shall be completed and final results submitted to EPA within 53 months of the test sponsor's receipt of a certified letter from EPA specifying that an oncogenicity test for HBCD be initiated.

(B) Progress reports shall be submitted to EPA every 6 months beginning 6 months after the effective date for PBDPE, OBDPE, and BTBPE and, for HBCD, beginning 6 months after the test sponsor's receipt of a certified letter specifying that an oncogenicity test be initiated until the final report is submitted.

(8) *Combined chronic toxicity/oncogenicity*—(i) *Required testing.* Combined chronic toxicity/oncogenicity tests shall be conducted in rats with PBDPE, OBDPE, and BTBPE by gavage, in accordance with § 798.3320 of this chapter.

(ii) *Reporting requirements.* (A) The combined chronic toxicity/oncogenicity testing shall be completed and the final reports submitted to EPA within 53 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 6 months after the effective date until the final report is submitted.

(d) *Environmental effects testing*—(1) *Algal testing* — (i) *Required testing.* Algal toxicity testing shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.1050 of this chapter. Algal toxicity testing shall be conducted with OBDPE and DBDPE in accordance with § 797.1050 of this chapter if an EC50 of $\leq$ 10 μg/L is obtained with PBDPE in this assay or, if that test concentration (EC50) is unattainable, at or below the limit of water solubility as determined by the water solubility testing conducted pursuant to paragraph (e)(1)(i) of this section.

(ii) *Reporting requirements.* (A) The algal toxicity test for PBDPE, BTBPE, and HBCD shall be completed and the final reports submitted to EPA within 15 months of the effective date. The algal toxicity test for OBDPE and DBDPE, if required, shall be completed and final results submitted to EPA within 24 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE and BTBPE, and beginning 21 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(2) *Fish chronic toxicity testing*—(i) *Required testing.* Fish early life stage toxicity tests shall be conducted with rainbow trout and sheepshead minnows with PBDPE, BTBPE, and HBCD in accordance with § 797.1600 of this chapter. Fish early life stage toxicity tests shall be conducted with rainbow trout and sheepshead minnows with OBDPE and DBDPE in accordance with § 797.1600 of this chapter if a geometric mean MATC value of $<$ 10 μg/L is obtained with PBDPE in this test with either fish species or, if that test concentration (geometric mean MATC value) is unattainable, at or below the limit of water solubility as determined by the water solubility testing conducted pursuant to paragraph (e)(1)(i) of this section.

(ii) *Reporting requirements.* (A) The fish early life stage toxicity tests for PBDPE, BTBPE, and HBCD shall be completed and the final reports submitted to EPA within 18 months of the effective date. The fish early life stage toxicity test for OBDPE and DBDPE, if required, shall be completed and the final reports submitted to EPA within 30 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD, and beginning 24 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(3) *Invertebrate chronic toxicity testing*—(i) *Required testing.* (A) Daphnid chronic toxicity tests shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.1330 of this chapter. A daphnid chronic toxicity

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test with OBDPE and DBDPE shall also be conducted in accordance with § 797.1330 of this chapter if a geometric an MATC of ≦ 10 μg/L is obtained h PBDPE in either this test or the mysid shrimp chronic toxicity tests conducted pursuant to paragraph (d)(3)(i)(B) of this section or, if that test concentration (geometric mean MATC) is unattainable, at or below the limit of water solubility as determined by the water solubility testing conducted pursuant to paragraph (e)(1)(i) of this section.

(B) Mysid shrimp chronic toxicity test shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.1950 of this chapter. Mysid shrimp chronic toxicity tests with OBDPE and DBDPE shall also be conducted in accordance with § 797.1950 of this chapter, if a geometric mean MATC of ≦ 10 μg/L is obtained with PBDPE in either this test or the daphnid chronic toxicity test conducted pursuant to paragraph (d)(3)(i)(A) of this section or, if that test concentration (geometric mean MATC value) is unattainable, at or below the limit of water solubility as determined by the water solubility testing conducted pursuant to paragraph (e)(1)(i) of this section.

(ii) *Reporting requirements.* (A) Invertebrate chronic toxicity testing all be conducted and the final reports mitted to EPA as follows:

(*1*) Daphnid chronic toxicity with PBDPE, BTBPE, and HBCD, 18 months after the effective date and, if required with OBDPE and DEDPE, 30 months after the effective date.

(*2*) Mysid shrimp chronic toxicity with PBDPE, BTBPE, and HBCD, 15 months after the effective date and, if required with OBDPE and DBDPE, 24 months after the effective date.

(B) Progress reports shall be as submitted to EPA as follows:

(*1*) For daphnid chronic toxicity, every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD and, if required, beginning 24 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(*2*) For mysid shrimp chronic toxicity, every 6 months beginning 9 months after the effective date for PBDPE, BTBPE, and HBCD, and if required, beginning 21 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(4) *Benthic organism chronic toxicity testing* —(i) *Required testing.* Chironomid sediment toxicity tests shall be conducted with PBDPE, BTBPE, and HBCD as specified in § 795.135 of this pter. Chironomid sediment toxicity s shall be conducted with OBDPE and DBDPE in accordance with § 795.135 of this chapter if a geometric mean MATC of ≦ 100 mg PBDPE/kg dry weight of sediment is obtained with PBDPE in this test.

(ii) *Reporting requirements.* (A) Chironomid sediment toxicity testing for PBDPE, BTBPE, and HBCD shall be completed and the final reports submitted to EPA within 18 months of the effective date. Chironomid sediment toxicity testing for OBDPE and DBDPE, if required, shall be completed and the final reports submitted to EPA within 30 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD and, if required, beginning 24 months after the effective date for OBDPE and DBDPE until a final report is submitted.

(5) *Terrestrial organism testing*—(i) *Required testing.* (A) Mallard reproduction tests shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.2150 of this chapter. This test shall also be conducted with OBDPE and DBDPE in accordance with § 797.2150 of this chapter if a no-observed-effect-level (NOEL) ≦ 500 ppm is obtained with PBDPE in this test.

(B) Earthworm soil subchronic toxicity tests shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 795.150 of this chapter. This test shall also be conducted with OBDPE and DBDPE in accordance with § 795.150 of this chapter if an EC50 of ≦ 100 mg PBDPE/kg dry weight of sediment is obtained with PBDPE in this test.

(ii) *Reporting requirements.* (A) Terrestrial organism testing shall be conducted and the final reports submitted to EPA as follows:

(*1*) Mallard reproduction testing with PBDPE, BTBPE, and HBCD and, if required with OBDPE and DBDPE, 30 months after the effective date.

(*2*) Earthworm toxicity testing with PBDPE, BTBPE, and HBCD, 18 months after the effective date and with OBDPE and DBDPE, 30 months after the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD and, if required, beginning 24 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(6) *Terrestrial plant testing*—(i) *Required testing.* (A) Seed germination/root elongation toxicity tests shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.2750 of this chapter. Seed germination/root elongation toxicity tests shall be conducted with OBDPE and DBDPE in accordance with § 797.2750 of this chapter if an EC50 of ≦ 100 mg PBDPE/kg dry weight of soil is obtained with PBDPE in either this test or the early seedling growth toxicity test conducted pursuant to paragraph (d)(6)(i)(B) of this section.

(B) Early seedling growth toxicity tests shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.2800 of this chapter. Early seedling growth toxicity tests shall be conducted with OBDPE and DBDPE in accordance with § 797.2800 of this chapter if an EC50 of ≦ 100 mg PBDPE/kg dry weight of soil is obtained with PBDPE in either this test or the seed germination/root elongation toxicity test is conducted pursuant to paragraph (d)(6)(i)(A) of this section.

(ii) *Reporting requirements.* (A) Terrestrial plant testing shall be conducted and the final reports submitted to EPA as follows:

(*1*) Seed germination/root elongation toxicity test with PBDPE, BTBPE, and HBCD, 15 months after effective date and, if required with OBDPE and DBDPE, 24 months after effective date;

(*2*) Early seedling growth toxicity test with PBDPE, BTBPE, and HBCD, 15 months after effective date and, if required with OBDPE and DBDPE, 24 months after effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD and, if required, every 6 months beginning 21 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(7) *Immunotoxicity*—(i) *Required testing.* (A) Immunotoxicity tests shall be conducted with PBDPE, BTDPE, and HBCD.

(B) The testing shall be conducted in accordance with the test procedure specified in an article by N.K. Jerne, et. al., entitled "Plaque Forming Cells: Methodology and Theory—I. The Standard Theory", published in *Transplant Reviews* 18:130–191 (1974), and in an article by M.I. Luster et. al., entitled "Methods Evaluation-Development of a Testing Battery to Assess Chemical-Induced Immunotoxicity: National Toxicology Program's Guidelines for Immunotoxicity Evaluation in Mice", published in *Fundamental and Applied Toxicology*, Vol. 10, pp. 2–19. (1988), which are incorporated by reference. Copies of these materials are available in the TSCA Public Reading Room, Rm. NE-G004, 401 M St., SW., Washington, DC 20460. These materials are also

available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC 20408. These orporations by reference were pproved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. These methods are incorporated as they exist on the effective date of this rule and a notice of any changes to the methods will be published in the **Federal Register**

(C) Immunotoxicity tests shall also be conducted with OBDPE and DBDPE in accordance with the methodology incorporated by reference in paragraph (d)(7)(i)(B) of this section, if a no observed effect level of ≦ 500 ppm is obtained with PBDPE in this test.

(ii) *Reporting requirements.* (A) Immunotoxicity tests for PBDPE, BTBPE, and HBCD shall be conducted and the final reports submitted to EPA within 15 months of the effective date. Immunotoxicity testing for OBDPE and DBDPE, if required, shall be completed and the final results submitted to EPA within 24 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD and, if required, beginning 18 months after the effective date for OBDPE and DBDPE until the final report is submitted.

3) *Bioconcentration*—(i) *Required testing.* Fish bioconcentration tests shall be conducted with PBDPE, BTBPE, and HBCD in accordance with § 797.1520 of this chapter except for the provisions in paragraphs (c)(1)(i), (c)(1)(iii), (c)(4)(ii)(A), (c)(4)(iii)(A), (c)(4)(iii)(B)(2), (c)(4)(iii)(C), (c)(4)(viii)(A), (c)(5)(i)(C), (c)(5)(i)(D), and (c)(5)(ii)(A) . Fish bioconcentration tests shall be conducted with OBDPE and DBDPE in accordance with § 797.1520, except for the provisions in paragraphs (c)(1)(i), (c)(1)(iii), (c)(4)(ii)(A), (c)(4)(iii)(A), (c)(4)(iii)(B)(2), (c)(4)(iii)(C), (c)(4)(viii)(A), (c)(5)(i)(C), (c)(5)(i)(D), and (c)(5)(ii)(A), if a bioconcentration factor of ≧ 1000 is obtained with PBDPE with this test.

(A) For purposes of this section, the following also applies:

(*1*) *Test procedures*—(*i*) *Summary of the test.* Fish are continuously exposed to at least two constant sublethal concentrations of a test substance under flow-through conditions for a maximum of 91 days. During this time, test solutions and fish are periodically sampled and analyzed using appropriate methods to quantify the test substance concentrations. The maximum depuration period is 56 days.

(*i*) If steady-state is not reached during 91 days of uptake, the steady-state BCF is calculated using non-linear parameter estimation methods.

(*2*) *Definitive test.* (*i*) At least two concentrations should be tested to assess the propensity of the compound to bioconcentrate. The concentrations selected should not stress or adversely affect the fish. The highest concentration should be less than the limit of water solubility. The lowest concentration should be one-tenth of the higher concentration, as long as that concentration is greater than three times the limit of quantification. The limiting factor for how low one can test is based on the detection limit of the analytical methods. The lower concentration of the test material in the test solution should be at least three times greater than the detection limit in water.

(*ii*) An estimate of the length of the uptake and depuration phases should be made prior to testing. This will allow the most effective sampling schedule to be determined. The uptake phase should continue for 91 days.

(*iii*) The following sampling schedule should be used to generate the appropriate data.

SAMPLING SCHEDULE (DAYS)

Sampling	Treatment	Controls
Exposure	1	1
	7	7
	14	–
	28	28
	56	–
	91	91
Depuration	7	7
	14	14
	28	–
	56	56

(*iv*) The depuration phase shall continue until at least 95 percent of the accumulated test substance and metabolites have been eliminated, but no longer than 56 days.

(*v*) At each of the designated sampling times, triplicate water samples and enough fish should be collected from the exposure chamber(s) to allow for at least three fish tissue analyses. A similar number of control fish should also be collected at each sample point, but only fish collected at the first sampling period and on days 1, 7, 28, and 91 for exposure treatment samples, and on days 7, 14, and 56 of depuration treatment samples should be analyzed. Triplicate control water samples will be collected at the time of test initiation and weekly thereafter. Test solution samples should be removed from the approximate center of the water column.

(*vi*) If steady-state was not reached during the 91 day uptake period, the maximum BCF should be calculated using the mean tissue concentration from that day and the mean water concentration from that and the previous sampling day. An uptake rate constant should then be calculated using appropriate techniques, such as the BIOFAC program developed by Blau and Agin (1978). This rate constant will allow the estimation of a steady-state BCF and the estimated time to steady-state.

(*vii*) If 95 percent elimination has not been observed after 56 days depuration, then a depuration rate constant should be calculated. This rate constant will allow estimation of the time to 95 percent elimination.

(*viii*) All samples shall be analyzed using gas chromatography coupled to a mass spectrometer (GC/MS) to quantitate each polybrominated biphenyl ether (PBBE) isomer present. All tests shall be conducted at aqueous concentrations below the measured water solubility of the specific mixture being tested. All tests shall be performed using samples from the identical commercial mixture. The specific methodology used shall be validated before the test is initiated. The accuracy of the method should be measured by the method of known additions. This involves adding a known amount of the test substance to three water samples taken from an aquarium containing dilution water and a number of fish equal to that to be used in the test. The nominal concentration of these samples shall be the same as the concentration to be used in the test. Samples taken on two separate days shall be analyzed: The accuracy and precision of GC/MS analytical method should be verified using reference samples or split samples or suitable corroborative methods of analysis. The accuracy of the standard solution should be checked against other standard solutions whenever possible.

(B) [Reserved]

(ii) *Reporting requirements.* (A) Fish bioconcentration tests for PBDPE, BTBPE, and HBCD shall be conducted and the final reports submitted to EPA within 18 months of the effective date. Fish bioconcentration tests for OBDPE and DBDPE, if required, shall be completed and the final reports submitted to EPA within 30 months of the effective date.

(B) Progress reports shall be submitted to EPA every 6 months beginning 12 months after the effective date for PBDPE, BTBPE, and HBCD, and, if required, beginning 24 months after the effective date for OBDPE and DBDPE until the final report is submitted.

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(e) *Chemical fate testing*—(1) *Water solubility*—(i) *Required testing.* (A) Water solubility tests shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with § 796.1860 of this chapter except for the provisions in paragraph (c)(1)(iii).

(B) For the purposes of this section the following provisions also apply:

(*1*) *Performance of the test.* (*i*) Determine the water solubility of the test compound in dilution water at the salinity and temperature specified for the algal toxicity test conducted pursuant to paragraph (d)(1)(i) of this section, for the fish early life stage toxicity tests conducted pursuant to paragraph (d)(2)(i) of this section, for the invertebrate chronic toxicity tests conducted pursuant to paragraphs (d)(3)(i)(A) and (d)(3)(i)(B) of this section, and for the benthic organism toxicity testing conducted pursuant to paragraph (d)(4)(i) of this section.

(*ii*) Water solubility shall be analyzed utilizing an electron capture detector.

(*2*) [Reserved]

(ii) *Reporting requirements.* The water solubility tests shall be completed and the final reports submitted to EPA within 6 months of the effective date.

(2) *Octanol/water partitioning*—(i) *Required testing.* Log octanol/water partition coefficient tests shall be conducted with PBDPE, OBDPE, and DBDPE in accordance with § 796.1720 of this chapter.

(ii) *Reporting requirements.* The log octanol/water partition coefficient tests shall be completed and the final reports submitted to EPA within 6 months of the effective date.

(3) *Vapor pressure*—(i) *Required testing.* Vapor pressure tests shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with § 796.1950 of this chapter.

(ii) *Reporting requirements.* The vapor pressure tests shall be completed and the final reports submitted to EPA within 6 months of the effective date.

(4) *Sediment and soil adsorption*—(i) *Required testing.* Sediment and soil adsorption tests shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with § 796.2750 of this chapter.

(ii) *Reporting requirements.* The sediment and soil adsorption tests shall be completed and the final reports submitted to EPA within 6 months of the effective date.

(5) *Photolysis*—(i) *Required testing.* Direct and indirect photolysis tests shall be conducted on congenerically pure PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with §§ 796.3780, 796.3800 and 796.3700 of this chapter.

(ii) *Reporting requirements.* The direct and indirect photolysis tests shall be completed and the final reports submitted to EPA within 6 months of the effective date.

(6) *Aerobic biodegradation*—(i) *Required testing.* (A) For each respective test substance, biodegradation testing in sediment/water shall be conducted with PBDPE, BTBPE, and HBCD.

(B) The testing shall be conducted using clean, freshwater sediments in accordance with the method described in an A.W. Bourquin article entitled "An Artificial Microbial Ecosystem for Determining Effects and Fate of Toxicants in a Salt-Marsh Environment", published in *Developments in Industrial Microbiology*, Vol. 18, Chapter 11, 1977, which is incorporated by reference. Copies of this material incorporated by reference are available in the TSCA Public Reading Room, Rm. NE–G004, 401 M St., SW., Washington, DC 20460. These materials are also available for inspection at the Office of the Federal Register, Rm 8401, 1100 L St., NW., Washington, DC 20408. This incorporation by reference was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. This method is incorporated as it exists on the effective date of this rule and notice of any change to the method will be published in the Federal Register.

(C) Biodegradation testing in sediment/water shall also be conducted with OBDPE and DBDPE in accordance with the methodology incorporated by reference in paragraph (e)(6)(i)(B) of this section, if mineralization to CO_2 greater than 10 percent is obtained with PBDPE in this test.

(ii) *Reporting requirements.* (A) Biodegradation testing in sediment/water shall be completed and the final reports submitted to EPA within 12 months of the effective date for PBDPE, BTBPE, and HBCD, and, if required, within 24 months of the effective date for OBDPE and DBDPE.

(B) Progress reports for biodegradation testing in sediment/water shall be submitted to EPA every 6 months beginning 6 months after the effective date for PBDPE, BTBPE, and HBCD, and, if required, every 6 months, beginning 18 months after the effective date for OBDPE and DBDPE until the final report is submitted.

(7) *Anaerobic biodegradation*—(i) *Required testing.* Anaerobic biodegradation testing shall be conducted with PBDPE, OBDPE, DBDPE, BTBPE, and HBCD in accordance with § 796.3140 of this chapter.

(ii) *Reporting requirements.* The anaerobic biodegradation test shall be completed and the final reports submitted to EPA within 6 months of the effective date.

(f) *Reference(s).* For additional background information, the following reference(s) should be consulted.

(1) Spenser, P.S., Eischoff, M.C., and Schaumburg, H.H. "Neuropathological methods for the detection of neurotoxic disease." In: *Experimental and Clinical Neurotoxicology*. P.S. Spenser and H.H. Schaumburg, eds. Baltimore, MD; Williams and Wilkins, pub. pp 743–757 (1980).

(2) [Reserved]

(g) *Effective date.* This section is effective (44 days after publication of the final rule in the Federal Register). (Information collection requirements have been approved by the Office of Management and Budget under OMB Control Number 2070–0033.)

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